(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification[7]:   C07K 14/35, 16/28, 16/46, 19/00, C12N 5/20, 15/13, A61K 39/04, 39/395, A61P 37/02

(21) International Application Number:
PCT/AU2003/001392

(22) International Filing Date: 20 October 2003 (20.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/420,232   18 October 2002 (18.10.2002)   US

(71) Applicant *(for all designated States except US)*: CENTE-NARY INSTITUTE CANCER MEDICINE & CELL BIOLOGY [AU/AU]; Royal Prince Alfred Hospital, Building 93, Missenden Road, Camperdown, NSW 2050 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BRITTON, War-wick [AU/AU]; 38 Stotts Avenue, Bardwell Park, NSW 2207 (AU). DEMANGEL, Caroline [FR/FR]; 14, rue du Tage, F-75013 Paris (FR).

(74) Agent: BALDWIN SHELSTON WATERS; 60 Margaret Street, Sydney, NSW 2000 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS FOR TARGETING ANTIGEN-PRESENTING CELLS WITH ANTIBODY SINGLE-CHAIN VARIABLE REGION FRAGMENTS

(57) **Abstract:** Provided are single-chain Fv (scFv) fragment-based compositions and methods for targeting antigens to antigen-presenting cells (APCs) such as, for example, dendritic cells (DC). Compositions and methods disclosed herein are useful in the treatment of diseases including infectious diseases and cancer.

- 1 -

COMPOSITIONS AND METHODS FOR TARGETING ANTIGEN-PRESENTING CELLS WITH ANTIBODY SINGLE-CHAIN VARIABLE REGION FRAGMENTS

5   BACKGROUND OF THE INVENTION

Technical Field of the Invention

The present invention relates generally to the fields of immunology and molecular biology. More specifically, the present invention is directed to antibody single-chain
10   variable region fragment (scFv)-based compositions and methods for targeting antigens to antigen-presenting cells (APCs) such as, for example, dendritic cells (DCs). Compositions and methods disclosed herein are useful in the treatment of diseases including infectious diseases and cancers.

15   Description of the Related Art

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Immunization has proved one of the most cost effective strategies for the
20   improvement of human health. Most of the effective vaccines against bacterial, parasitic, and viral pathogens depend on the production of antibodies. Protective immunity against a number of important human and veterinary pathogens depends, however, upon the development of cellular immune responses. In addition, application of therapeutic and prophylactic immunization methodology to vaccines directed against cancers also depends
25   upon the stimulation of cellular immune responses to vaccine components. Accordingly, effective strategies for eliciting cellular immunity will prove widely applicable to the development of vaccines against infectious diseases and cancers.

The *in vivo* processes involved in the development of cellular immunity continue to be more clearly delineated. One class of antigen-presenting cell, the dendritic cell (DC), is
30   critical in sensing the presence of foreign organisms that play a central role in the induction of antimicrobial immunity. Scattered throughout the body, they constitute the first line of

- 2 -

defence against invading pathogens. Innate immune recognition by DCs is based on the recognition of microbial motifs by specialised receptors, the identification of which is a field of growing interest. Moll, *Cellular Microbiology* 5:493-500 (2003); Figdor *et al.*, *Nature Reviews Immunology* 2:77-84 (2002); and Demangel *et al.*, *Immunology & Cell Biology*

5   78:318-324 (2000). Following interaction with antigen, DC undergo a maturation process resulting in the up-regulation of expression of co-stimulatory, adhesion and MHC molecules enhancing their capacity to present peptides to naïve T cells.

DCs migrate to specialized lymphoid organs, the lymph nodes, to stimulate immunity and undergo maturation to become effective antigen-presenting cells capable of

10   stimulating T lymphocytes (T-cells). This process has been studied in mycobacterial infections such as TB. Infection of DC by *M. tuberculosis* or BCG induces the co-ordinate processes of DC maturation and secretion of the cytokine interleukin 12 (IL-12). These events are critical in the development of mycobacteria-specific T-cells.

DCs represent a minor cell subset of the peripheral tissues. In steady state conditions,

15   lung DCs constitute less than 1% of the total cell population, a low incidence rate considering their sentinel role against incoming pathogens. Moll *et al.*, *Cellular Microbiology* 5:493-500 (2003). This sparse distribution is compensated for by a high sensitivity to environmental signals, delivered by damaged endogenous tissues or by pathogens. Austyn, *Nature Medicine* 5:1232-3 (1999). Microbial products (cell wall

20   components, non-methylated CpG motifs, double stranded RNA) are potent inducers of DC activation. Sousa *et al.*, *Current Opinion in Immunology* 11:392-399 (1999). Moreover, model antigens expressed in recombinant bacteria are presented by MHC Class I and Class II molecules on DC much more efficiently than the same antigens in soluble form. Svensson *et al.*, *J. Immunol.* 158:4229-36 (1997); Rescigno *et al.*, *Proc. Natl. Acad. Sci.*

25   *USA* 95:5229-34 (1998). This strongly suggests that enhanced antigen presentation could be achieved by selective targeting of subunit vaccines to the DC receptors, which are specialized in the recognition of bacterial products.

Immature DCs display receptors on their surface membranes that permit them to bind to and, in some cases, internalize a diverse array of antigens. Internalized protein-

30   antigens are processed into short peptides that are presented in the context of MHC Class I

- 3 -

and Class II molecules. Following the interaction of DC receptors with antigens, DC undergo a maturation that results in the increased expression of co-stimulatory and MHC molecules that enhance their capacity to present peptides to naïve T-cells.

A number of receptor molecules have been identified on the surface of DCs. DEC-205, a homologue of the macrophage mannose receptor, and the integrin CD11c are surface receptors that are restricted to DCs. Immunological evidence suggests that targeting antigens to DEC-205 or to CD11c may improve antigen presentation by DCs. Thus, it is likely that DEC-205 and CD11c play an important role in antigen capture. Rat antibodies directed to mouse DEC-205 are more efficiently internalized than non-specific rat antibodies and are 100-fold more effective at generating T-cell responses to the anti-DEC-205 antibody than to the non-specific rat antibodies. Jiang et al., Nature 375:151-5 (1995). Similar results have been obtained by immunizing mice with anti-CD11c antibodies.

The β2 integrin CD11c is an attractive candidate for investigating the impact of antigen targeting to DCs because it is a DC-restricted surface molecule expressed by all subsets of mouse DCs and all human DCs of myeloid origin. Wilson et al., Immunology and Cell Biology 81:239-246 (2003); and Pulendran et al., Trends in Immunology 22:41-7 (2001). Although its function is still unclear, there is immunological evidence that CD11c is involved in antigen capture and delivery to antigen processing compartments. Finkelman et al., J. Immunol. 157:1406-1414 (1996). So is DEC-205, a lectin receptor expressed by mouse DC subpopulations of the spleen, Peyer's patches, lymph nodes and skin, and by some human DC subsets. Anjuere et al., Blood 93:590-8 (1999) and Guo et al., Human Immunology 61:729-738 (2000). Despite significant sequence homology with the macrophage mannose receptor (MMR) and the presence of eight C-type carbohydrate recognition domains, DEC-205 does not bind mannose and its specific ligands have yet to be defined. Jiang et al., Nature 375:151-5 (1995). Both MMR and DEC-205 receptors mediate adsorptive uptake of antigen in coated vesicles, direct antigen loaded vesicles to the endosomal compartment end recycle to the cell surface. However, whereas MMR recycles through early endosomes, DEC-205 targets antigens to the MHC Class II rich late endosomal compartment, leading to enhanced antigen presentation to CD4$^+$ T cells. Guo et

- 4 -

al., Human Immunol. 61:729-738 (2000). Improving the delivery of antigens to DEC205 or CD11c receptors may thus result in enhanced T cell priming by DC.

Antigen targeting to sites of immune induction is an efficient means of enhancing immune responses to DNA vaccines. Directing antigens to B7-expressing cells using
5 cytotoxic T-lymphocyte antigen-4 (CTLA4) promotes the development of immune responses to fusion antigen in mice. Boyle *et al., Nature* 392:408-11 (1998). B7 molecules are expressed by a broad spectrum of leukocytes, including professional antigen presenting cells such as DCs, but also B and T lymphocytes. Products fused to L-selectin, a lymphocyte surface molecule mediating cell entry in the lymph nodes, are less efficient than
10 the CTLA-4 fused ones in promoting T-cell proliferative responses suggesting that selective antigen targeting to cell subsets specialised in antigen presentation is more effective for immune stimulation. The stimulatory effect of scNLDC may also relate to the fact that DEC-205-endocytosis pathway is highly efficient for antigen presentation to $CD4^+$ T cells. Mahnke *et al., J. Cell Biol.* 151:673-683 (2000).

15 Protein antigen targeting to DEC-205 using chemically-coupled antibody molecules has been shown to induce T cell unresponsiveness *in vivo* under steady state conditions. Hawiger *et al., J. Exp. Med.* 194:769-779 (2001) and Bonifaz *et al., J. Exp. Med.* 196:1627-1638 (2002). Tolerance was, however, converted into prolonged T cell stimulation if the antigen was co-administered with an additional stimulus (such as an anti-CD40 antagonist).

20 Tuberculosis (TB) is an intracellular bacterial infection, the control of which is dependent upon cellular immunity. TB remains the single most prevalent bacterial infection world-wide, with one third of the world's population currently being infected with *Mycobacterium tuberculosis*. From this pool of 2 billion infected individuals, 8-9 million new cases of clinical tuberculosis develop a year resulting in the death of at least 2 million
25 people. Because of the interaction of *M. tuberculosis* and HIV, about half the deaths associated with HIV/AIDS in developing countries occur because of active tuberculosis. The meta-analysis of clinical trials with the only currently available vaccine, *M. bovis* Bacille Calmette Guerin (BCG), has led to the conclusion that BCG confers about 50% protective efficacy against the common pulmonary form of tuberculosis. This level of

- 5 -

efficacy has proven insufficient to control the spread of tuberculosis and underscores the need for new immunization strategies.

Despite the progress that has been made in identifying receptors and other molecules on the surface of APCs and DCs, there remains a need in the art for improved compositions and methods for the delivery of antigens to APCs and DCs in order to achieve improved therapeutic and prophylactic efficacy against diseases including infectious diseases, autoimmune diseases, and cancers.

SUMMARY OF THE INVENTION

The present invention addresses these and other related needs by providing, *inter alia*, compositions and methods for targeting antigen-presenting and dendritic cells with antigens, including protein-antigens. As disclosed herein, compositions and methods will find utility in the treatment of disease by enhancing the cellular immune response to antigens.

Disclosed herein are single chain antibody fragments (scFvs) from the monoclonal antibodies NLDC-145 and N418, which are directed to DEC-205 and CD11c mouse DC receptors. Exemplary scFv presented herein have the typical structure of scFvs, with the variable domain of the immunoglobulin heavy chain (V_H) linked to the light chain one (V_L) via a flexible peptide linker in a V_H-V_L orientation. Nissim *et al.*, *EMBO J.* 13:692-698 (1994). These scFvs bind to their target receptor comparably to the parental antibodies *in vitro*. Thus, scFv targeting, as provided herein, is a powerful means for eliciting strong immune responses *in vivo*.

Within certain embodiments, the present invention provides antibody single-chain variable region fragments (scFv) for targeting antigen-presenting cells (APCs) such as, for example, dendritic cells (DC). scFv presented herein comprise an antibody heavy chain variable region (V_H) operably linked to an antibody light chain variable region (V_L) wherein the heavy chain variable region and the light chain variable region together or individually form a binding site for specifically binding to a molecule on the surface of an APC and/or a DC. ScFv may comprise a V_H region at the amino-terminal end and a V_L region at the

- 6 -

carboxy-terminal end. Equally suitable are scFv that comprise a V$_L$ region at the amino-terminal end and a V$_H$ region at the carboxy-terminal end.

An exemplary scFv is derived from monoclonal antibody NLDC-145 which antibody specifically binds to DEC-405 on the surface of DC. According to this embodiment, the

5   scFv comprises variants of the NLDC-145 heavy chain (V$_H$) and light chain (V$_L$) variable regions wherein each variant NLDC-145 heavy chain (V$_H$) and light chain (V$_L$) region is at least 70%, 80%, 90%, 95% or 98% identical to the sequences disclosed herein in SEQ ID NOs: 5 and 6, respectively. A most preferred exemplary scFv, disclosed herein in SEQ ID NO: 7, comprises the NLDC-145 heavy chain (V$_H$) and light chain (V$_L$) variable regions

10 disclosed herein in SEQ ID NOs: 5 and 6, respectively.

An alternative preferred exemplary scFv is derived from monoclonal antibody N418 which antibody specifically binds to CD11c on the surface of DC. According to this embodiment, the scFv comprises variants of the N418 heavy chain (V$_H$) and light chain (V$_L$) variable regions wherein the variant N418 derived scFv is at least 70%, 80%, 90%, 95% or

15 98% identical to the sequences disclosed herein in SEQ ID NO: 2. A most preferred exemplary scFv comprises the N418 heavy chain (V$_H$) and light chain (V$_L$) variable regions which scFv is disclosed herein in SEQ ID NOs: 2.

ScFv disclosed herein may, optionally, further comprise a polypeptide linker operably linked between the heavy chain variable region and the light chain variable region.

20 Polypeptide linkers of the present invention generally comprise between 1 and 50 amino acids. More common are polypeptide linkers of at least 2 amino acids. Even more commonly, polypeptide linkers are between 3 and 12 amino acids. An exemplary linker peptide for incorporating between scFv heavy and light chains comprises the 5 amino acid sequence Gly-Gly-Gly-Gly-Ser. Alternative exemplary linker peptides comprise one or

25 more tandem repeats of the sequence Gly-Gly-Gly-Gly-Ser to create linkers comprising, for example, the sequences Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser.

Surface molecules on APC and/or DC that may be targeted by scFv of the present

30 invention include proteins and carbohydrates. Within certain embodiments, surface protein

- 7 -

molecules include receptor proteins. Surface receptor proteins may facilitate internalization of the specifically bound scFv into the APC and/or the DC. Within certain aspects, specifically bound scFv may be internalized by receptor-mediated endocytosis and/or by pinocytosis. Preferred surface protein molecules include, but are not limited to, the

5   mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1 (CD80), B7-2 (CD86), CD40, CD11c, DEC-205, a Toll-like receptor (TLR), and the Fcγ receptor (FcγR). Most preferred are those surface protein molecules that are restricted to DCs such as CD11c and DEC-205.

Other aspects of the present invention provide complexes between scFv and one or more antigens, including protein-antigens. Antigens encompass protein-antigens that

10   undergo *in vivo* post-translational modifications wherein the protein-antigen may be glycosylated, lipidated, phosphorylated or the like.

Further aspects of the present invention provide complexes comprising scFv and a lipid. Thus, exemplified herein are scFv-lipid complexes wherein the scFv comprises a tag such as an affinity tag. Suitable affinity tags include, but are not limited to, the FLAG-tag

15   and the hexahistidine tag. Thus, for example, a hexahistidine tagged scFv may form a complex directly with a lipid, such as a metal chelating lipid. An exemplary metal chelating lipid presented herein is nitrilotriacetic acid ditetradecylamine (NTA-DTDA).

Within still further aspects of the present invention, scFv may be complexed directly with a lipid and/or with one or more antigen that is encapsulated by, incorporated within,

20   and/or associated with a lipid membrane, a lipid bi-layer, and/or a lipid complex such as, for example, a liposome, a vesicle, a micelle and/or a microsphere. Thus, within these aspects of the invention, the term "antigen" encompasses such liposomes, vesicles, micelles and/or microspheres that comprise an antigen, such as a protein-antigen, including glycoprotein-antigens and/or lipoprotein-antigens.

25   Complexes between scFv, a lipid, and/or an antigen may be achieved by chemical crosslinking or, alternatively, may be a fusion protein comprising scFv heavy and light chain variable regions and an antigen. Suitable scFv that may be employed in the complexes comprising an scFv, such as scFv/antigen, scFv/lipid, and scFv/lipid/antigen complexes, include those indicated above and as described in further detail herein below. scFv/antigen

- 8 -

complexes are capable of specifically binding to APC and/or DC thereby facilitating the targeting of the antigen to the APC and/or DC.

An exemplary scFv/antigen complex presented herein is the scFv NLDC-145-85B encoded by the nucleotide sequence presented herein as SEQ ID NO: 8. Equally preferred

5   are functional fragments, derivatives and variants of the scFv NLDC-145-85B encoded by the nucleotide sequence presented herein as SEQ ID NO: 8. Functional variants of scFv NLDC-145-85B preferably exhibit at least about 70%, more preferably at least about 80% or 90% and most preferably at least about 95% or 98% sequence identity to the polypeptide encoded by SEQ ID NO: 8.

10 Another exemplary scFv/antigen complex presented herein is the scFv N418-85B encoded by the nucleotide sequence presented herein as SEQ ID NO: 3. Equally preferred are functional fragments, derivatives and variants of the scFv N418-85B encoded by the nucleotide sequence presented herein as SEQ ID NO: 3. Functional variants of scFv N418-85B typically exhibit at least about 70%, more typically at least about 80% or 90% and most

15 typically at least about 95% or 98% sequence identity to the polypeptide encoded by SEQ ID NO: 3.

Within certain aspects, antigens that may be complexed with the inventive scFv include protein-antigens from an organism, including a virus, parasite or a bacterium, which is capable of causing an infectious disease in a human. Exemplary viral organisms include,

20 but are not limited to, human immunodeficiency virus (HIV), a herpes virus, and an influenza virus. Exemplary parasitic organisms include, but are not limited to, *Leishmania (e.g., L. major* and *L. donovani)*. Exemplary bacterial organisms include, but are not limited to, *Mycobacteria (e.g., M. tuberculosis* and *M. bovis)*, *Chlamydia (e.g., C. trachomatis* and *C. pneumoniae)*, and *Ehrlichia (e.g., E. sennetsu, E. chaffeensis, E. ewingii,* and *E.*

25 *phagocytophila)*. Within certain aspects, the protein-antigen is an *M. tuberculosis* antigen selected from the group consisting of 85B, MPT64, and ESAT-6 disclosed herein in SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18, respectively. Other aspects provide that the protein-antigen is a fragment, derivative or variant of 85B, MPT64, or ESAT-6. Typical protein-antigens exhibit at least about 70%, more typically at least about 80% or 90% and

- 9 -

most typically at least about 95% or 98% sequence identity to the polypeptide disclosed herein in SEQ ID NO: 14, SEQ ID NO: 16, and/or SEQ ID NO: 18.

The present invention also provides fusion proteins, comprising an antigen-presenting cell binding protein and a protein-antigen wherein the fusion protein is capable of
5   specifically binding to an antigen-presenting cell (APC) and/or a dendritic cell (DC) and in inducing a protein-antigen specific T-cell response. According to certain aspects, the APC and/or DC binding protein specifically binds to a receptor on the APC and/or DC. Exemplary receptors include, but are not limited to, the mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1, B7-2, CD40, CD11c, DEC-205, a Toll-like receptor (TLR), and
10   the Fcγ receptor (FcγR). Preferred antigens are infectious disease antigens, autoimmune disease antigens, or cancer cell antigens, including tissue-specific and/or tumor-specific antigens, as indicated above and as described in further detail herein.

Further aspects of the present invention provide polynucleotides that encode one or more of the scFv presented herein. Within certain embodiments, the polynucleotide is a
15   component of a vector, such as a plasmid vector or a viral vector, wherein the vector comprises a transcriptional promoter operably linked to the scFv encoding polynucleotide.

Related aspects provide polynucleotides that encode an scFv/antigen fusion protein which polynucleotides comprise a first polynucleotide that encodes an scFv and a second polynucleotide that encodes one or more protein-antigen wherein the first polynucleotide
20   and the second polynucleotide are operably linked such that together they encode a fusion protein comprising an scFv and a protein-antigen. More preferred embodiments provide that the first polynucleotide and the second polynucleotide are operably linked by a third polynucleotide that encodes a polypeptide linker between the scFv and the protein-antigen. Within certain embodiments, the polynucleotide encoding the scFv/protein antigen fusion
25   protein is a component of a vector, such as a plasmid vector or a viral vector, wherein the vector comprises a transcriptional promoter operably linked to the scFv encoding polynucleotide. Particularly preferred vectors comprising a polynucleotide encoding an scFv and an scFv/antigen are, respectively, the pcDNA3-NLDC-145 and pcDNA3-NLDC-85 plasmid vectors presented herein in Figure 1 as well as pcDNA3-N418-85. The
30   nucleotide sequences encoding scFv NLDC-145-85B, scFv N418-85B, and the nucleotide

- 10 -

sequence of pcDNA3 are presented herein in SEQ ID NO: 8, SEQ ID NO: 3, and SEQ ID
NO: 9, respectively.

The present invention also provides compositions comprising scFv, scFv/lipid,
scFv/antigen, and/or scFv/lipid/antigen complexes as well as compositions comprising
5   polynucleotides encoding scFv and/or scFv/antigen complexes and compositions comprising
vectors comprising one or more polynucleotides encoding an scFv and/or an scFv/antigen
complex. Exemplary compositions may, optionally, further comprise a cytokine such as
interleukin-12 (IL-12), IL-6, IL-4, IL-1, interferon-γ (IFNγ), GM-CSF, tumor necrosis factor
(TNF), and/or the CD40 ligand CD154, and/or may comprise a lipopolysaccharide (LPS) or
10   other inducer of the DC response to antigen, such as other cell wall components, non-
methylated CpG motifs, and/or double-stranded RNA.

Other aspects of the present invention provide methods for introducing an antigen
into an antigen-presenting cell (APC) and/or a dendritic cell (DC), the methods comprising
the steps of: (a) isolating from a patient sample, an APC and/or a DC; and (b) contacting
15   the APC and/or DC with an scFv/antigen complex, wherein the scFv/antigen complex is in
contact with the APC and/or DC under conditions and for such a time as required to permit
the antigen to enter the APC and/or DC.

Related aspects of the present invention provide methods for introducing an antigen
into an APC and/or a DC of a patient, the methods comprising the step of administering to a
20   patient a composition comprising an scFv/antigen complex, thereby inducing an interaction
with an APC and/or a DC of the patient.

Still further related aspects provide methods for introducing a protein-antigen into an
APC and/or a DC of a patient, the methods comprising the step of administering to the
patient a composition comprising a polynucleotide encoding an scFv/antigen complex.

25   Still further aspects of the present invention provide methods for treating a disease
and/or modulating an immune response in a patient, the methods comprising the steps of:
(a) obtaining from the patient a sample comprising an antigen-presenting cell (APC) and/or
a dendritic cell (DC); (b) contacting the sample with an scFv/antigen complex under
conditions and for such a time as required to allow binding of the scFv/antigen complex to
30   the APC and/or DC; and (c) administering the scFv/antigen APC and/or DC-bound complex

- 11 -

to the patient. Modulation of the immune response may include enhancing, stimulating, suppressing, and/or blocking the immune response in the patient.

Within methods for the present invention, the disease may be selected from the group consisting of an infectious disease, an autoimmune disease and a cancer. More

5   preferred methods provide that the infectious disease is caused by an organism selected from the group consisting of *Leishmania, Mycobacteria, Chlamydia,* and *Ehrlichia.* Equally preferred methods provide that the cancer is selected from the group consisting of soft tissue sarcomas, lymphomas, and cancers of the brain, esophagus, uterine, cervix, bone, lung, endometrium, bladder, breast, larynx, colon/rectum, stomach, ovary, pancreas, adrenal gland

10   and prostate.

Other aspects provide methods for inhibiting, reducing, suppressing and/or blocking the activity of a target antigen on the surface of an antigen-presenting cell (APC) and/or a dendritic cell (DC), the methods comprising the steps of: (a) obtaining a sample comprising and APC and/or a DC; (b) contacting the APC and/or DC with an scFv capable of

15   specifically binding to the target antigen on the surface of the APC and/or DC under conditions and for such a time as required to permit binding of the scFv to the APC and/or DC, wherein binding of the scFv to the APC and/or DC blocks or substantially reduces the activity of the target antigen, thereby inhibiting, reducing, suppressing and/or blocking an immune response.

20   By any of the methods disclosed herein, the scFv may bind to a molecule, including a carbohydrate molecule or a protein molecule, on the surface of the APC and/or DC. Preferred surface protein molecules include, but are not limited to, the mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1, B7-2, CD40, CD11c, DEC-205, a Toll-like receptor (TLR), and the Fcγ receptor (FcγR).

25   Within certain methods, the scFv may be complexed to an antigen wherein scFv/antigen complexes are achieved by chemical crosslinking or wherein scFv/antigen complexes are scFv/antigen fusion proteins.

Suitable antigens that may be employed in any of the methods disclosed herein include, but are not limited to, antigens from an organism, including a virus, a parasite, or a

30   bacterium, which is capable of causing an infectious disease in a human. Exemplary viral

- 12 -

organisms include, but are not limited to, human immunodeficiency virus (HIV), a herpes virus, and an influenza virus. Exemplary bacterial organisms include, but are not limited to, *Mycobacteria, Chlamydia,* and *Ehrlichia.* Exemplary parasitic organisms include, but are not limited to, *Leishmania.* Within certain aspects, the antigen is an *M. tuberculosis* antigen

5   selected from the group consisting of 85B, MPT64, and ESAT-6 disclosed herein in SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18, respectively.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was

10   incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE IDENTIFIERS

Figure 1 depicts plasmid maps for (A) pcDNA3-NLDC-145 expressing an scFv derived from anti-DEC-205 hybridoma NLDC-145, and (B) pcDNA3-NLDC-85 in which

15   the gene for the *M. tuberculosis* antigen 85B is fused to the anti-DEC-205 derived scFv.

Figure 2 depicts a plasmid map of DNA vectors used for transfections and immunizations exemplified within the examples disclosed herein. Two vectors were constructed in which the ScNLDC or ScN418 sequences were fused to the Ag85B gene via a 12 amino acid spacer. The scFv-Ag85B construct was linked 5' to HBM secretion

20   sequence and 3' to a FLAG detection sequence (*i.e.* Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys). Transcription of the fusion protein in mammalian cells was under the control of the human cytomegalovirus promoter (pCMV) and the bovine growth hormone transcription terminator BGHpA.

Figures 3A depicts the deduced amino acid sequence of V_H and V_L domains in

25   NLDC-145 and N418 monoclonal antibodies. Underlined sequences correspond to complementarity determining regions (CDR). Figure 3B presents the results of an analysis of culture supernatant from insect cells infected with recombinant baculoviruses expressing ScNLDC: Coomassie blue staining (1), Western blot using detection antibodies binding FLAG (2) and polyHis (3) peptides.

- 13 -

Figures 4A-4D present immunohistological data demonstrating that scNLDC binds Langerhans cells with the same specificity as the parental antibody. Epidermal sheets of mouse ears stained with NLDC-145 whole IgG molecule (A) or with purified ScNLDC, as detected via the C-terminal poly-Histidine tail (B) or FLAG peptide (D), were compared. A
5   control epidermis incubated with secondary reagents in the absence of ScNLDC is shown (C).

Figures 5A-5D present immunohistological data demonstrating that scN418 binds dendritic cells with the same specificity as the parental antibody. FSDCs stained with N418 whole IgG molecule (A) or with purified ScN418, as detected via the C-terminal poly-
10 Histidine tail (B) or FLAG peptide (D), were compared. Control cells incubated with secondary reagents only were included (C).

Figure 6 is a bar graph depicting induction of an Interferon-gamma (IFN-γ) T-cell response following *in vivo* administration of the pscNLDC-Ag85B, pAg85B, or the control vector pCDNA3 (ctrl). Mice were compared for the frequency of IFN-γ producing cells in
15 the spleen (A) and total IFN-γ production (B) following restimulation with purified Ag85B protein four weeks after single injection of DNA. Mean ($\pm$SE) in three mice groups are shown, and are representative of three independent experiments. Differences between groups were analyzed using ANOVA (*$p<0.05$, **$p<0.01$).

Figure 7 is a bar graph depicting induction of specific antibody response following *in*
20 *vivo* administration of mice with the pcDNA3-NLDC-85 and pcDNA3-85 vectors. Titers of Ag85B-specific serum IgG were compared in mice immunized with pscNLDC-Ag85B, pAg85B or the control vector pCDNA3 (ctrl), two and four weeks after injection of a single dose of DNA vaccine. The horizontal dotted line indicates the background level of the ELISA. Mean ($\pm$SE) in three mice groups are shown, and are representative of two
25 independent experiments.

Figure 8 is a bar graph depicting the protective effect of immunization of mice with pcDNA3-NLDC-85, pcDNA3-85 and the control pcDNA3 vectors and the currently used live vaccine BCG. C57BL/6 mice (n=5) were immunised by three intramuscular injections of 100 μg of each of the three DNA vaccines or 5×10^4 BCG by subcutaneous injection. The
30 bacterial counts of *M. tuberculosis* (mean $\pm$SD) in the lungs and spleens of mice (n=5) were

- 14 -

determined 4 weeks after aerosol infection with *M. tuberculosis*. The pcDNA3-NLDC-85 vaccine was significantly more effective than pcDNA-85 vaccine (p<0.05) and the control pcDNA3 vaccine (p<0.01) and there was no significant difference in the effect of the pcDNA3-NLDC-85 vaccine and BCG.

5   SEQ ID NO: 1 is the nucleotide sequence encoding scFv N418 of SEQ ID NO: 2.

SEQ ID NO: 2 is the amino acid sequence of scFv N418.

SEQ ID NO: 3 is the nucleotide sequence for scFv N418-85B.

SEQ ID NO: 4 is the nucleotide sequence encoding scFv NLDC145 of SEQ ID NO: 7.

10   SEQ ID NO: 5 is the deduced amino acid sequence of the heavy chain variable region (V_H) of the NLDC-145 monoclonal antibody.

SEQ ID NO: 6 is the deduced amino acid sequence of the light chain variable region (V_L) of the NLDC-145 monoclonal antibody.

SEQ ID NO: 7 is the amino acid sequence of scFv NLDC145.

15   SEQ ID NO: 8 is the nucleotide sequence for scFv NLDC-145-85B.

SEQ ID NO: 9 is the nucleotide sequence for pcDNA3 (Invitrogen; Carlsbad, California).

SEQ ID NO: 10 is the amino acid sequence of an exemplary linker peptide for incorporating between an scFv and an antigen in an scFv/antigen complex.

20   SEQ ID NO: 11 is the nucleotide sequence encoding the linker peptide of SEQ ID NO: 10.

SEQ ID NO: 12 is the nucleotide sequence for baculovirus vector pBACPak 8 (Genbank Accession No. U02446).

SEQ ID NO: 13 is the nucleotide sequence encoding *M. tuberculosis* antigen 85B

25   (Genbank Accession No. X62398).

SEQ ID NO: 14 is the amino acid sequence for *M. tuberculosis* antigen 85B (Genbank Accession No. CAA44269).

SEQ ID NO: 15 is the nucleotide sequence encoding *M. tuberculosis* antigen mpt64 (Genbank Accession No. X75361).

30   SEQ ID NO: 16 is the amino acid sequence for *M. tuberculosis* (H37Rv) antigen

- 15 -

MPT64 (Genbank Accession No. NP_216496).

SEQ ID NO: 17 is the nucleotide sequence encoding *M. tuberculosis* antigen esat-6 (Genbank Accession No. AF420491).

SEQ ID NO: 18 is the amino acid sequence for *M. tuberculosis* antigen ESAT-6
5   (Genbank Accession No. Q57165).

DETAILED DESCRIPTION OF THE INVENTION

As indicated above, the present invention is directed to antibody single-chain variable region fragment (scFv)-based compositions and methods for targeting antigen-
10   presenting cells (APCs) such as, for example, dendritic cells (DC). Disclosed herein are scFv-based complexes, such as scFv/lipid, scFv/antigen, and scFv/lipid/antigen complexes, which specifically bind to molecules on the surface of APC and/or DC and, in the case of scFv/antigen complexes, are suitable for introducing the antigen into the APC and/or DC. Complexes of the present invention may be employed to enhance and/or stimulate T-cell
15   responses to candidate antigen and, as exemplified herein, known antigens of *M. tuberculosis* such as 85B. Inventive scFv-based complexes may be used to enhance and/or stimulate the immune response in the patient thereby reducing the severity of the infectious diseases, including diseases caused by *mycobacterial* infections such as tuberculosis.

Also disclosed herein are methods employing the inventive scFv-based complexes
20   which methods are suitable for blocking the activity of a target antigen on the surface of an APC; for introducing antigens, either *ex vivo* or *in vivo*, into APC; for modulating, stimulating and/or inhibiting an immune response in a patient; as well as for treating a disease in a patient such as an infectious disease, an autoimmune disease, and a cancer. Without being limited to a particular mode of action, the methods disclosed herein may
25   facilitate T-cell priming for antibody production and may provide an effective mechanism for increasing antibody responses to recombinant protein-antigens. In addition, the combined effect of increasing T-cell and antibody responses to antigens may be particularly applicable to tissue-specific and tumor-specific antigens that are associated with cancers.

Each of these aspects of the present invention is described in further detail herein
30   below.

- 16 -

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the context clearly dictates otherwise.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive

5   sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods for virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described

10   below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, *et al.*, "Molecular Cloning: A Laboratory Manual" (2nd Edition, 1989); Maniatis *et al.*, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach, vol. I & II" (D. Glover, ed.); "Oligonucleotide Synthesis" (N. Gait, ed., 1984); "Nucleic Acid Hybridization" (B. Hames & S. Higgins, eds., 1985); "Transcription

15   and Translation" (B. Hames & S. Higgins, eds., 1984); "Animal Cell Culture" (R. Freshney, ed., 1986); and Perbal, "A Practical Guide to Molecular Cloning" (1984). All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

20   <u>Antigen-presenting Cell (APC)- and Dendritic Cell (DC)-specific Molecules</u>

As noted above, the present invention provides single-chain variable region fragment (scFv)-based complexes and compositions and methods employing such complexes for targeting antigen-presenting cells (APCs), including dendritic cells (DCs). Each of the inventive scFv that are disclosed, and employed in the inventive complexes, compositions

25   and methods, specifically bind to a carbohydrate and/or protein molecule on the surface of an APC and/or a DC.

As used herein, the term "specifically bind" or "specifically binding" refers to the ability of an antibody, and/or an scFv derived from that antibody, to detect a target molecule or single epitope out of a population of non-target molecules on the surface of an antigen-

30   presenting cell such as a dendritic cell. Specific binding may be determined by a number of

- 17 -

methods available in the art including, for example, assays based on primary interactions between an antibody and/or scFv and the corresponding target molecule. Exemplary assays for measuring primary interactions include radioimmunoassay (RIAs) and enzyme-linked immunosorbent assays (ELISAs). In addition, specific binding may be determined by

5   measuring secondary interactions such as by measuring changes in the physical and/or biochemical properties of the target antigen that occur as a consequence of contacting the target antigen with an antibody and/or an scFv. For example, secondary interactions may be measured by immunoprecipitation of a labeled target antigen followed by detection of the label or by detection of a reaction, such as autophosphorylation, catalyzed by the

10   immunoprecipitated antigen.

The term "antigen-presenting cell" or "APC" refers to those highly specialized cells that can process antigens and display their peptide fragments on the cell surface together with molecules required for lymphocyte activation. The main antigen-presenting cells for T-cells are DC, macrophages, and B-cells, whereas the main antigen-presenting cells for B-

15   cells are follicular dendritic cells.

The term "dendritic cell" or "DC" is defined as those APCs that are found in T-cell areas of lymphoid tissues. Banchereau *et al.*, *Nature* <u>392</u>:245-251 (1998). DCs are a sparsely distributed, migratory group of bone-marrow-derived leukocytes that are specialized for the uptake, transport, processing and presentation of antigens to T-cells.

20   Non-lymphoid tissues also contain DCs, but these do not stimulate T-cell responses until they are activated and migrate to lymphoid tissues. In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes, dendrites, visible *in vitro*); their ability to take up, process and present antigens with high efficiency; and their ability to activate naïve T-cell responses. DCs of the present invention

25   are distinct from the follicular DC that present antigens to B-cells. For a general review of murine and human dendritic cells, see Shortman *et al.*, *Nat. Rev. Immunol.* <u>2(3)</u>:151-61 (2002).

Exemplary surface molecules on APC and/or DC that may be targeted by the scFv of the present invention are receptor molecules including, but not limited to, the mannose

30   receptor (MR), chemokine receptor 1 (CCR1), B7-1 (CD80), B7-2 (CD86), CD40, CD11c,

- 18 -

DEC-205, a Toll-like receptor (TLR), and the Fcγ receptor (FcγR). Most preferred are those surface protein molecules that are restricted to DCs such as CD11c and DEC-205. Other embodiments of the present invention provide scFv that bind specifically to carbohydrates and/or carbohydrates attached to APC and/or DC-specific surface molecules.

The mannose receptor (MR) is expressed by macrophages and DC and recognizes carbohydrate groups such as mannose or fucose that are exposed on a number of microorganisms, including mycobacteria. Sallusto *et al., J. Exp. Med.* 182:389-400 (1995); Engering *et al., Adv. Exp. Med. Biol.* 417:183-7 (1997); Engering *et al., Eur. J. Immunol.* 27:2417-25 (1997); Prigozy *et al., Immunol.* 6:187-97 (1997); and Tan *et al., Adv. Exp. Med. Biol.* 417:171-4 (1997). Mannose receptors on macrophages recycle constitutively and may thus allow antigen internalization in successive rounds. Because ligand binding to MR induces the release of pro-inflammatory cytokines such as IL-1, IL-6 and IL-12 by DC, MR engagement may act by promoting both antigen presentation and DC maturation, and therefore further facilitate T-cell stimulation. Yamamoto *et al., Infect. Immun.* 65:1077-82 (1997) and Shibata *et al., J. Immunol.* 159:2462-7 (1997).

CCR1 is the main receptor expressed by immature DC and is downregulated on LPS- or TNF-mediated activation of DC. Because immature DC are more efficient at capturing and processing antigens than are mature DC, it may be advantageous to target scFv/antigens of the present invention to immature DC by utilizing scFv that specifically bind to CCR1.

B7-1 (CD80) and B7-2 (CD86) are co-stimulatory glycoprotein molecules expressed on APC. The B7 molecules are homodimeric members of the immunoglobulin superfamily found exclusively on the surface of cells capable of stimulating T-cell growth. These molecules bind to CD28 on T-cells to co-stimulate the growth of naïve T-cells. CD80 (B7-1) is expressed on monocytes, immature dendritic cells and activated B cells and T cells. It is important in the regulation of T cell activation and is a ligand for CD28 and CD152 (CTLA-4). CD86 (B7-2) is expressed on interdigitating dendritic cells and monocytes, upregulated on recirculating B cells following activation, germinal B cells and memory B cells. CD86 is a coreceptor for CD28 and CD152 (CTLA-4).

- 19 -

CD40 is a transmembrane protein expressed on APC including macrophages and B-cells. CD40 is found on normal and neoplastic B-cells Hodgkin and Reed-Sternberg cells, normal basal epithelial and epithelial cell carcinomas, interdigitating cells (IDC), marcophages, follicular dendritic cells, fibroblasts keratinocytes and some endothelial cells.

5   Ligation of CD40 on B-cells mediates diverse outcomes depending on the stage of differentiation and the epitope engaged. CD40 plays a central role in developing and promoting events associated with T-cell differentiation and antibody responses. Ligation of CD40 on macrophages induces them to secrete TNF-α and to become receptive to reduced concentrations of IFN-γ while ligation of CD40 on B-cells promotes growth and antibody

10   isotype switching.

CD11c is a DC restricted integrin that, similar to DEC-205, induces a strong T-cell immune response when stimulated with anti-CD11c antibodies. Finkelman *et al., J. Immunol.* 157:1406-14 (1996). CD11c is able to recognize several microbial substances, including bacterial lipopolysaccharide (LPS), the lipophosphoglycan of *Leishmania*, the

15   filamentous hemagglutinin of *Bordetella*, and structures on yeasts such as *Candida* and *Histoplasma*.

DEC-205 is a macrophage mannose receptor-related C lectin that is restricted in expression to DCs and is involved in antigen processing. Witmer-Pack *et al., Cell. Immunol.* 163:157-62 (1995); Inaba *et al., Cell. Immunol.* 163:148-56 (1995); U.S. Patent

20   No. 6,117,977 and U.S. Patent No. 6,046,158. The multilectin domain structure of DEC-205 suggests that it may enable DC to bind highly diverse carbohydrate-bearing antigens. Antigen targeting to DEC-205 may improve antigen presentation by DC, indicating the potential for DEC-205 to capture and deliver antigen to processing compartments.

The Toll-like receptors (TLRs) on mammalian cells are able to detect a variety of

25   microbial components and, consequently, are a major component of the innate immunity to microbial infections. Anderson, *Curr. Opin. Immunol.* 12:13-19 (2000). Mycobacterial lipoproteins appear to stimulate IL-12 on human macrophages through Toll-like receptor 2 (TLR2). Brightbill *et al., Science* 285:732-6 (1999). TLRs may participate in the induction of primary responses to mycobacteria by DC. Demangel *et al., Immunol.* and *Cell Biol.*

30   78:318-324 (2000).

- 20 -

The FC gamma receptor (FCγR) is expressed on DC. FCγR binds to the constant region of immunoglobulins of the IgG isotype and induces endocytosis of the immune complexes. Cella *et al.*, *Curr. Opin. Immunol.* 9:10-16 (1997).

Preferred APC- or DC-specific molecules facilitate the specific binding and/or
5   introduction of an inventive scFv, scFv/lipid complex, scFv/antigen complex, and/or scFv/lipid/antigen complex into the APC or DC by a process of internalization such as, for example, receptor-mediated endocytosis or pinocytosis and, most preferably, enable the display of peptides derived from the *in vivo*-processing of the antigen on the cell-surface within the context of MHC Class I or MHC Class II molecules.

10
Single-chain Fv (scFv)

As noted above, the present invention provides antibody single-chain Fv (scFv) scFv-based complexes, including, but not limited to, scFv/lipid, scFv/antigen, and scFv/lipid/antigen complexes wherein the scFv specifically binds to a molecule on the
15   surface of an APC and/or a DC. As used herein, the terms "single-chain Fv" and "scFv" refer to recombinant proteins comprising an antibody heavy chain variable (V_H) region operably linked to an antibody light chain variable (V_L) region. Optionally, scFv further comprise a "linker" peptide that serves as a spacer between the heavy chain variable region and the light chain variable region. In addition, scFv may comprise a signal (or leader)
20   sequence at the N-terminal end that co-translationally or post-translationally directs transfer of the protein and/or may comprise a tag, such as an affinity tag, exemplified by the FLAG-tag and hexahistidine tag, to facilitate complex formation such as scFv/antigen, scFv/lipid, and/or scFv/lipid/antigen complex formation.

ScFv constructs of the present invention provide numerous advantages over whole
25   antibody-based therapeutics. Because scFvs are produced in substantial quantities and with minimal purification requirements, such as in a baculovirus expression system, they represent an economical alternative to whole antibody molecules. Moreover, scFvs may be administered repeatedly without inducing deleterious host immune responses against the Fc part of the immunoglobulin chains.

30   A large array of antigens and lipids can be potentially directed to DCs using the scFv

- 21 -

constructs of the present invention. For example, protein antigens may be fused to scFvs by genetic engineering methodologies, and potentially any kind of compounds may be chemically joinable to the scFv via its affinity tag.

Single-chain Fv may be generated by a number of methodologies that are readily

5   available in the art. Most commonly, scFv are generated from hybridomas that express a monoclonal antibody having the desired antigen binding specificity and affinity. For example, scFv of the present invention may be generated from hybridomas that express monoclonal antibodies that specifically bind to a molecule that is exposed on the surface of an APC and/or a DC. Exemplified herein are scFv that were generated from hybridomas,

10   designated NLDC-145 and N418, that express monoclonal antibodies that specifically bind to DEC-205 and CD11c, respectively, on the surface of dendritic cells.

Polynucleotides encoding antibody heavy and light chain variable regions may be amplified from total hybridoma cell RNA. For example, first-strand cDNA may be synthesized using reverse transcriptase and random hexamers. Heavy and light chain

15   variable regions may then be amplified from the cDNA by utilizing primer pairs that hybridize 5' and 3' to each of the heavy and light chain variable region coding regions. See, for example, U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159. Primer sequences suitable for PCR amplification of scFv heavy and light chains are disclosed in U.S. Patent No. 6,248,516 and PCT Patent Application Publication No. WO 90/05144.

20   Polynucleotides isolated in this way may be combined by utilizing conventional recombinant DNA methodology such that the polynucleotide comprising the V_H coding region is fused in-frame with the polynucleotide comprising the V_L coding region. Depending on the precise scFv to be expressed, it may be desirable to fuse the V_H coding region 5' to the V_L coding region. Alternatively, the V_H coding region may be fused 3' to

25   the V_L coding region. Regardless of the orientation, in-frame fusion of the V_H and V_L coding regions permits translation into a single scFv protein that retains the biological activity of the component V_H and V_L polypeptides. (For general guidance on the design of scFv, see U.S. Patent No. 4,946,778).

A polynucleotide encoding a peptide linker sequence may be employed to separate

30   the encoded V_H and V_L regions by a distance sufficient to ensure that each polypeptide folds

- 22 -

into a functional secondary and tertiary structure. Such a peptide linker sequence is incorporated into the fusion protein using standard recombinant DNA techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) the ability of the linker to adopt a flexible extended conformation; and (2) the

5 lack of hydrophobic or charged residues that might react with the antigen binding sites on, or created by, the V_H and V_L regions. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. A particularly preferred peptide linker exemplified herein comprises three tandem repeats of the five amino acid sequence Gly-Gly-Gly-Gly-Ser to generate the

10 15 amino acid linker having the amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser.

Depending on the particular application contemplated, it may be desired to design the scFv to favor the formation of a multimer such as a dimer, trimer, and/or tetramer of the monomeric scFv. For example, within the context of tumor targeting, where scFv having

15 molecular weights in the 60-100 kDa range have been shown to exhibit increased tumor penetration and faster clearance rates compared to the parent immunoglobulin, it may be preferred to form scFv dimers (~ 60 kDa), trimers (~90 kDa) or tetramers (~120 kDa). Kortt *et al.*, *Biomol. Eng.* 18(3):95-108 (2001).

ScFv multimers may be achieved by varying the length of the polypeptide linker that

20 joins the heavy chain variable region to the light chain variable region. Amino acid sequences that may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46 (1985); Murphy *et al.*, *Proc. Natl. Acad. Sci.* USA 83:8258-8262 (1986); U.S. Pat. No. 4,935,233; and U.S. Pat. No. 4,751,180.

The polypeptide linker may generally be from 1 to about 50 amino acids in length.

25 ScFv joined with a polypeptide linker of at least 12 amino acids predominantly forms monomers while scFv joined with a linker of 3-11 amino acids may be sterically prohibited from folding into a monomeric form and, instead, associate with a second scFv to form a dimer. scFv joined with linkers of less than 3 amino acids may form predominantly trimers or tetramers depending upon the linker length, composition and scFv variable region

30 orientation.

- 23 -

Within certain embodiments, scFv will be encoded by a polynucleotide that comprises a first polynucleotide encoding a V_H region and a second polynucleotide encoding a V_L region. Polynucleotides encoding preferred scFv further comprise a third polynucleotide that encodes a linker of at least 1 amino acid, preferable at least 3 amino

5   acids. More preferred third polynucleotides encode linkers of between 3 and 11 amino acids. Most preferred third polynucleotides encode linkers of at least 12 amino acids.

Within still further embodiments, one or more polynucleotide encoding an affinity tag may be operably linked either 5' and/or 3' to a polynucleotide encoding an scFv of the present invention. As exemplified herein, suitable affinity tags include, but are not limited

10   to, hexahistidine (i.e. His-His-His-His-His-His-), or multiples thereof, and the FLAG-tag (i.e. Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys). When expressed in the context of an scFv polypeptide, such affinity tags may be usefully employed in detection assays, utilizing, for example, Ni or an epitope-specific antibody in the case of hexahistidine and the FLAG-tag, respectively. Affinity tags may alternatively be utilized to facilitate the direct interaction

15   between an APC- and/or DC-specific scFv, including but not limited to an anti-DEC205 or an anti-CD11c scFv as disclosed herein, and a lipid moiety, such as a metal-chelating lipid as, for example, nitrilotriacetic acid ditetradecylamine (NTA-DTDA) as presented within PCT Patent Application Publication Nos. WO00064471 and WO09855853, each of which patent application is incorporated by reference herein in its entirety.

20   The ligated polynucleotide sequences may be operably linked to suitable transcriptional or translational regulatory elements to achieve expression and translation of the scFv *ex vivo* or *in vivo*. The regulatory elements responsible for expression of the scFv coding region are generally located 5' to the polynucleotide sequence encoding the amino-terminal V_H or V_L region. Similarly, stop codons required to end translation and

25   transcription termination signals may be present 3' to the polynucleotide encoding the carboxy-terminal V_H or V_L region.

It will be appreciated that scFv of the present invention may be employed in methods for targeting antigens, including protein-antigens, to APC and/or DC. Suitable antigens include antigens from a wide variety of bacterial, parasitic and/or viral organisms, as

30   indicated elsewhere herein. Accordingly, vectors expressing scFv may be engineered to

- 24 -

accommodate the in-frame fusion of polynucleotides encoding antigens of any organism.
For example, standard recombinant DNA methodology may be employed to introduce one
or more cloning site immediately 3' to the scFv coding region to facilitate the convenient,
in-frame subcloning into the vector of an antigen encoding polynucleotide. The

5   scFv/antigen fusion protein resulting from expression of the fusion construct will find utility
in targeting the antigen to a surface molecule, such as a receptor molecule, on an antigen-
presenting and/or dendritic cell.

Antigens Utilized in scFv/Antigen Complexes

10   As described above, scFv of the present invention may be employed to introduce one
or more antigens into an antigen-presenting cell such as a dendritic cell. Within certain
embodiments are provided complexes between scFv, that specifically bind to APC and/or
DC, and antigens such as, for example, infectious disease antigens, autoimmune disease
antigens, and cancer antigens, including tissue-specific antigens and/or tumor-specific

15   antigens.
            As used herein, the term "antigen" as used in the context of scFv/antigen complexes
broadly encompasses such antigens as protein-antigens, including glycoprotein-antigens,
lipoprotein-antigens, and phosphoprotein-antigens. For example, it will be understood that
scFv/antigen complexes, such as scFv/antigen fusion proteins, may undergo *in vivo* post-

20   translational modifications wherein the protein-antigen may be glycosylated, lipidated,
phosphorylated or the like. Within certain embodiments of the present invention, scFv may
be complexed with one or more antigen that is encapsulated by, incorporated within, and/or
associated with a lipid membrane, a lipid bi-layer, and/or a lipid complex such as, for
example, a liposome, a vesicle, a micelle and/or a microsphere. Within such embodiments,

25   therefore, the term "antigen" encompasses such liposomes, vesicles, micelles and/or
microspheres that comprise an antigen, such as a protein-antigen, including glycoprotein-
antigens and/or lipoprotein-antigens.
            Thus, the present invention contemplates scFv/antigen complexes wherein the
antigen is a protein-antigen encoded by a polynucleotide obtained from a virus, parasite or

30   bacterium that is a causative agent of an infectious disease. Provided are protein-antigens

- 25 -

encoded by polynucleotides from viral organisms including, but not limited to, human immunodeficiency virus (HIV), a herpes virus, and an influenza virus. Also provided are protein-antigens from parasitic organisms including, but not limited to, *Leishmania (e.g., L. major* and *L. donovani)* and from bacterial organisms including, but not limited to,

5 *Mycobacteria (e.g., M. tuberculosis* and *M. bovis), Chlamydia (e.g., C. trachomatis* and *C. pneumoniae),* and *Ehrlichia (e.g., E. sennetsu, E. chaffeensis, E. ewingii,* and *E. phagocytophila).*

Exemplified herein are scFv/antigen complexes comprising scFv that specifically bind to the DC-restricted surface receptor molecules DEC-205 or CD11c, and the protein-

10 antigen 85B from *Mycobacteria tuberculosis.* It has previously been shown that a DNA vaccine expressing 85B (pcDNA.85) induces protective cellular immune responses against aerosol infection with *M. tuberculosis* in mice. Palendira *et al., Infection and Immunity* 70(4):1949-1956 (2002) and U.S. Patent No. 6,384,018. As part of the present invention, a polynucleotide encoding 85B was fused in-frame with and to the 3'-end of a polynucleotide

15 encoding a scFv constructed from the anti-DEC-205 hybridoma NLDC-145.

Within certain aspects, the protein-antigen is an *M. tuberculosis* antigen selected from the group consisting of 85B, MPT64, and ESAT-6 disclosed herein in SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18, respectively. Other aspects provide that the protein-antigen is a fragment, derivative or variant of 85B, MPT64, or ESAT-6. Preferred variants

20 of the protein-antigens 85B, MPT64, or ESAT-6 exhibit at least about 70%, more preferably at least about 80% or 90% and most preferably at least about 95% or 98% sequence identity to the polypeptide disclosed herein in SEQ ID NO: 14, SEQ ID NO: 16, and/or SEQ ID NO: 18.

Equally suited antigens for preparing scFv/antigen complexes of the present

25 invention include extracellular *mycobacterial* antigens, disclosed in U.S. Patent No. 5,108,745 and the 79 kDa antigen of *M. bovis* Bacille Calmette Guerin (BCG), disclosed in U.S. Patent Nos. 6,045,798 and 5,330,754. Other antigens that may be employed in scFv/antigen complexes included the immunostimulatory peptides presented in U.S. Patent Nos. 6228,371, 6,214,543, 6,087,163, and 4,889,800.

- 26 -

Additionally, U.S. Patent No. 6,060,259 discloses *Mycobacterium* protein-antigens, in particular those of *M. bovis*, having molecular weights between approximately 44.5 and 47.5 kDa that may be employed in scFv/antigen complexes of the present invention. U.S. Patent No. 5,840,855 provides 540 and 517 amino acid protein-antigens, and corresponding
5   polynucleotides, from *Mycobacterium tuberculosis*.

ScFv/antigen Complexes

Within certain embodiments, the present invention provides complexes between scFv and antigens, including protein-antigens and between scFv and lipids, such as metal
10 chelating lipids. Such complexes may be achieved by any methodology available in the art. Most commonly, scFv/antigen complexes are formed through chemical means, such as by conventional coupling techniques, or are expressed as fusion proteins encoded by polynucleotides that encode antibody heavy and light chain variable regions. Other embodiments of the present invention provide that scFv/antigen complexes may further
15 comprise one or more lipid moiety to create scFv/lipid/antigen complexes.

For example, any of the scFv disclosed herein may be chemically coupled to an antigen using a dehydrating agent such as dicyclohexylcarbodiimide (DCCI) to form a bond, such as a peptide bond between the scFv and the antigen. Alternatively, linkages may be formed through sulfhydryl groups, epsilon amino groups, carboxyl groups or other reactive
20 groups present in the antigens, using commercially available reagents. (Pierce Co., Rockford, Illinois).

As noted above, scFv of the present invention may also be complexed with one or more antigen that is encapsulated by, incorporated within, and/or associated with a lipid membrane, a lipid bi-layer, and/or a lipid complex such as, for example, a liposome, a
25 vesicle, a micelle and/or a microsphere. Within such embodiments, complex formation may be achieved by cross-linking the scFv to the liposome, vesicle, micelle and/or microsphere following standard methodology that is readily available in the art. See, *e.g.*, Metselaar *et al.*, *Mini Rev. Med. Chem.* 2(4):319-29 (2002) and references cited therein. Suitable methods for preparing lipid-based antigen delivery systems that may be employed with the
30 scFv of the present invention are described in O'Hagen *et al.*, *Expert Rev. Vaccines*

- 27 -

2(2):269-83 (2003); O'Hagan, *Curr. Durg Targets Infect. Disord.* 1(3):273-86 (2001); Zho *et al.*, *Biosci Rep.* 22(2):355-69 (2002); Chikh *et al.*, *Biosci Rep.* 22(2):339-53 (2002); Bungener *et al.*, *Biosci. Rep.* 22(2):323-38 (2002); Park, *Biosci Rep.* 22(2):267-81 (2002); Ulrich, *Biosci. Rep.* 22(2):129-50; Lofthouse, *Adv. Drug Deliv. Rev.* 54(6):863-70 (2002);

5   Zhou *et al.*, *J. Immunother.* 25(4):289-303 (2002); Singh *et al.*, *Pharm Res.* 19(6):715-28 (2002); Wong *et al.*, *Curr. Med. Chem.* 8(9):1123-36 (2001); and Zhou *et al.*, *Immunomethods* 4(3):229-35 (1994).

Depending upon the precise application contemplated, scFv of the present invention may be complexed with one or more lipid and/or lipid encapsulated antigen through an

10   affinity tag such as, for example, hexahistidine or a FLAG-tag and described herein above. According to such exemplary embodiments, a metal-chelating lipid may be employed such as, for example, nitrilotriacetic acid ditetradecylamine (NTA-DTDA) as presented within PCT Patent Application Publication Nos. WO00064471 and WO09855853, each of which patent application is incorporated by reference herein in its entirety.

15   Equally suited to the practice of the present invention are scFv/antigen complexes expressed as fusion proteins comprising an scFv operably linked with an antigen. scFv/antigen fusion proteins may be prepared using conventional recombinant DNA methodology wherein the 3'-end of a first polynucleotide encoding an scFv is ligated in-frame with the 5'-end of a second polynucleotide encoding one or more protein-antigen.

20   Accordingly, the first polynucleotide and the second polynucleotide are operably linked such that they encode a fusion protein comprising the scFv and one or more protein-antigens.

More preferred embodiments provide that the first polynucleotide and the second polynucleotide are operably linked by a third polynucleotide that is ligated in-frame between the 3'-end of the first polynucleotide and the 5'-end of the second polynucleotide such that a

25   polypeptide linker is encoded between the scFv and the protein-antigen coding regions.

Within certain embodiments, the polynucleotide encoding the scFv/antigen fusion protein is a component of a vector, such as a plasmid vector or a viral vector, for facilitating expression of the fusion protein. Preferably, the vector comprises a transcriptional promoter operably linked 5' to the scFv encoding polynucleotide and a translational stop and/or

30   transcription termination signal 3' to the protein-antigen(s) coding region.

- 28 -

Exemplary vectors comprising a polynucleotide encoding inventive scFvs include the pBCV/NLDC-145 baculovirus expression vector described in Example 1 and the pcDNA3-NLDC-145 plasmid vector presented in Figure 1A. An exemplary vector comprising a first polynucleotide encoding an scFv (anti-DEC-205 or anti-CD11c) and a

5   second polynucleotide encoding the *mycobacterial* protein-antigen 85B is the pcDNA3-NLDC-85 and pcDNA3-N418-85 plasmid vectors presented herein in Figure 1B and described in the Examples. The nucleotide sequence of scFv NLDC-85, scFv N418-85, and pcDNA3 are presented herein in SEQ ID NO: 8, SEQ ID NO: 3, and SEQ ID NO: 9, respectively.

10   Expression may be achieved in any appropriate host-cell that has been transformed or transfected with an expression vector that contains the necessary elements for transcription and translation and that contains a polynucleotide encoding an scFv or scFv/antigen of the present invention. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are bacterial (*E. coli*), yeast,

15   insect, or a mammalian cell line such as COS or CHO.

In general, scFv-based complexes (whether formed by crosslinking, as fusion proteins, and/or by coupling to a lipid moiety) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. Preferably, complexes are at least about 90% pure, more preferably at

20   least about 95% pure and most preferably at least about 99% pure.

ScFv and scFv/antigen complexes may be isolated from culture supernatants by utilizing suitable host cell/vector systems that secrete the scFv or scFv/antigen fusion proteins into culture media. For example, total protein may be concentrated using a commercially available filter and applied to a suitable purification matrix such as an affinity

25   matrix or an ion exchange resin. One or more chromatography steps may be employed to further purify a recombinant polypeptide. In addition, scFv and/or scFv/antigen fusion proteins may further utilize a polypeptide affinity tag, such as hexahistidine (*i.e.* His-His-His-His-His-His-), or multimers thereof, and the FLAG tag polypeptide exemplified herein (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys), to facilitate detection and/or purification of the scFv

30   or scFv/antigen fusion protein from the culture supernatant. Such affinity tags may also be

- 29 -

employed to facilitate complex formation between scFv and/or scFv/antigen complexes and a lipid, such as a metal chelating lipid.

<u>ScFv and scFv/antigen Fragments, Derivatives and Variants</u>

It will be appreciated that scFv and scFv/antigen complexes according to the present invention encompass fragments, derivatives, and variants of either or both of the heavy and light chain variable regions and/or the antigen so long as the fragments, derivatives, and variants do not substantially affect the functional properties of the scFv and/or the antigen.

A polypeptide or protein "fragment, derivative, and variant," as used herein, is a polypeptide or protein that differs from a native polypeptide or protein in one or more substitutions, deletions, additions and/or insertions, such that the functional activity of the polypeptide or protein is not substantially diminished. In other words, the ability of a variant to specifically bind to an antigen-presenting cell (APC) and/or a dendritic cell (DC) surface molecule or to be internalized and/or processed by the APC and/or DC may be enhanced or unchanged, relative to the scFv and/or antigen, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein, without affecting the efficacy of the resulting scFv and/or scFv/antigen complex.

Such fragments, derivatives, and variants may generally be identified by modifying amino acid sequence of the scFv V_H and/or V_L moiety and evaluating the reactivity of the modified scFv with APC and/or DC or with antisera raised against the native protein-antigen. Such modification and evaluation may be achieved through routine application of molecular and cell biology techniques that are well known in the art.

Polypeptide fragments, derivatives, and variants preferably exhibit at least about 70%, more preferably at least about 80% or 90% and most preferably at least about 95% or 98% sequence identity to the native polypeptide or protein. Preferably, variants contain "conservative amino acid substitutions" as defined as a substitution in which one amino acid is substituted for another amino acid that has similar properties, such that the secondary structure and hydropathic nature of the polypeptide is substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example,

- 30 -

negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other

5   groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes.

Variants may additionally, or alternatively, be modified by, for example, the deletion or addition of amino acids that have minimal influence on the surface molecule specific

10   binding, secondary structure and hydropathic nature of the scFv and/or protein-antigen.

Functional fragments, derivatives, and variants of a polypeptide may be identified by first preparing fragments of the polypeptide by either chemical or enzymatic digestion of the polypeptide, or by mutation analysis of the polynucleotide that encodes the polypeptide and subsequent expression of the resulting mutant polypeptides. The polypeptide fragments or

15   mutant polypeptides are then tested to determine which portions retain biological activity, using, for example, the representative assays provided below.

Fragments, derivatives, and variants of the inventive polypeptides may also be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated

20   using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963). Equipment for automated synthesis of polypeptides is

25   commercially available from suppliers such as Perkin Elmer/Applied BioSystems, Inc. (Foster City, California), and may be operated according to the manufacturer's instructions. Variants of a native polypeptide may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed, site-specific mutagenesis. Kunkel, *Proc. Natl. Acad. Sci. USA* 82:488-492 (1985). Sections of polynucleotide sequence may also be removed using

30   standard techniques to permit preparation of truncated polypeptides.

- 31 -

As used herein, the term "variant" comprehends nucleotide or amino acid sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant sequences (polynucleotide or

5   polypeptide) preferably exhibit at least 70%, more preferably at least 80% or at least 90%, more preferably yet at least 95%, and most preferably, at least 98% identity to a sequence of the present invention. The percentage identity is determined by aligning the two sequences to be compared as described below, determining the number of identical residues in the aligned portion, dividing that number by the total number of residues in the inventive

10   (queried) sequence, and multiplying the result by 100. In addition to exhibiting the recited level of sequence similarity, variant sequences of the present invention preferably exhibit a functionality that is substantially similar to the functionality of the sequence against which the variant is compared.

Polynucleotide sequences may be aligned, and percentages of identical nucleotides in

15   a specified region may be determined against another polynucleotide, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. The alignment and identity of polypeptide sequences may be examined using the BLASTP algorithm. BLASTX and FASTX algorithms compare nucleotide query

20   sequences translated in all reading frames against polypeptide sequences. The FASTA and FASTX algorithms are described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444-2448 (1988); and in Pearson, *Methods in Enzymol.* 183:63-98 (1990). The FASTA software package is available from the University of Virginia by contacting David Hudson, Assistant Provost for Research, University of Virginia, P.O. Box 9025, Charlottesville, VA

25   22906-9025. The FASTA algorithm, set to the default parameters described in the documentation and distributed with the algorithm, may be used in the determination of polynucleotide variants. The readme files for FASTA and FASTX Version 2.0x that are distributed with the algorithms describe the use of the algorithms and describe the default parameters.

- 32 -

The BLASTN software is available on the NCBI anonymous FTP server and is
available from the National Center for Biotechnology Information (NCBI), National Library
of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894. The BLASTN algorithm
Version 2.0.6 [Sep-10-1998] and Version 2.0.11 [Jan-20-2000] set to the default parameters

5   described in the documentation and distributed with the algorithm, is preferred for use in the
determination of variants according to the present invention. The use of the BLAST family
of algorithms, including BLASTN, is described at NCBI's website and in the publication of
Altschul *et al.*, "Gapped BLAST and PSI-BLAST: a new generation of protein database
search programs," *Nucleic Acids Res.* 25:3389-3402 (1997).

10   The following running parameters are preferred for determination of alignments and
identities using BLASTN that contribute to the E values and percentage identity for
polynucleotides: Unix running command with the following default parameters: blastall -p
blastn -d embldb -e 10 -G 0 -E 0 -r 1 -v 30 -b 30 -i queryseq —o results; and parameters are:
-p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost

15   to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero
invokes default behavior) [Integer]; -r Reward for a nucleotide match (BLASTN only)
[Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to
show (B) [Integer]; -i Query File [File In]; -o BLAST report Output File [File Out]
Optional.

20   The "hits" to one or more database sequences by a queried sequence produced by
BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of
sequences. The hits are arranged in order of the degree of similarity and the length of
sequence overlap. Hits to a database sequence generally represent an overlap over only a
fraction of the sequence length of the queried sequence.

25   The percentage identity of a polynucleotide or polypeptide sequence is determined
by aligning polynucleotide and polypeptide sequences using appropriate algorithms, such as
LASTN or BLASTP, respectively, set to default parameters; identifying the number of
identical nucleic or amino acids over the aligned portions; dividing the number of identical
nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide

30   or polypeptide of the present invention; and then multiplying by 100 to determine the

- 33 -

percentage identity. By way of example, a queried polynucleotide having 220 nucleic acids has a hit to a polynucleotide sequence in the EMBL database having 520 nucleic acids over a stretch of 23 nucleotides in the alignment produced by the BLASTN algorithm using the default parameters. The 23-nucleotide hit includes 21 identical nucleotides, one gap and one

5   different nucleotide. The percentage identity of the queried polynucleotide to the hit in the EMBL database is thus 21/220 times 100, or 9.5%. The identity of polypeptide sequences may be determined in a similar fashion.

The BLASTN and BLASTX algorithms also produce "Expect" values for polynucleotide and polypeptide alignments. The Expect value (E) indicates the number of

10   hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion

15   of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a probability of 90% of being related. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN algorithm. E values for polypeptide sequences may be determined in a similar

20   fashion using various polypeptide databases, such as the SwissProt database.

According to one embodiment, "variant" polynucleotides and polypeptides, with reference to each of the polynucleotides and polypeptides of the present invention, preferably comprise sequences having the same number or fewer nucleic or amino acids than each of the polynucleotides or polypeptides of the present invention and producing an E

25   value of 0.01 or less when compared to the polynucleotide or polypeptide of the present invention. That is, a variant polynucleotide or polypeptide is any sequence that has at least a 99% probability of being related as the polynucleotide or polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or BLASTX algorithms set at the default parameters. According to a preferred embodiment, a variant

30   polynucleotide is a sequence having the same number or fewer nucleic acids than a

- 34 -

polynucleotide of the present invention that has at least a 99% probability of being related as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN algorithm set at the default parameters. Similarly, according to a preferred embodiment, a variant polypeptide is a sequence having the same number or fewer

5   amino acids than a polypeptide of the present invention that has at least a 99% probability of being related as the polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTP algorithm set at the default parameters.

In addition to having a specified percentage identity to an inventive polynucleotide or polypeptide sequence, variant polynucleotides and polypeptides preferably have

10   additional structure and/or functional features in common with the inventive polynucleotide or polypeptide. Polypeptides having a specified degree of identity to a polypeptide of the present invention share a high degree of similarity in their primary structure and have substantially similar functional properties. In addition to sharing a high degree of similarity in their primary structure to polynucleotides of the present invention, polynucleotides having

15   a specified degree of identity to, or capable of hybridizing to, an inventive polynucleotide preferably have at least one of the following features: (i) they contain an open reading frame or partial open reading frame encoding a polypeptide having substantially the same functional properties as the polypeptide encoded by the inventive polynucleotide; or (ii) they contain identifiable domains in common.

20   Suitable variants of the scFv NLDC-145-85B and scFv N418-85B disclosed herein comprise sequence variations within the amino acid sequences of the scFv and/or 85B moieties. For example, the present invention contemplates protein conjugates wherein the scFv NLDC-145-85B and scFv N418-85B are at least 70% identical with the amino acid sequences encoded by the polynucleotides recited in SEQ ID NOs: 8 and 3, respectively.

25   More preferred are scFv NLDC-145-85B and scFv N418-85B that are at least 80%, 90%, 95% and 98% identical to the amino acid sequences recited in SEQ ID NOs: 8 and 3, respectively.

- 35 -

Methods for Use

ScFv-based complexes of the present invention, including compositions thereof, will find utility in a number of methods as exemplified by those disclosed herein.

5   Within certain embodiments, the present invention provides, for example, *ex vivo* methods for introducing an antigen into an antigen-presenting cell (APC) and/or a dendritic cell (DC). By these methods, APC and/or DC are isolated from a patient sample and contacted with the isolated APC and/or DC with an scFv/antigen complex under conditions and for such a time as required to permit the antigen to enter the APC and/or DC.

10   Each of the *ex vivo* methods disclosed herein requires the isolation of antigen-presenting cells (APC) and/or dentritic cells (DCs) from a patient sample, most preferably a human. APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells. Dendritic cells and progenitors may be obtained from

15   peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. DCs may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or

20   bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allow a simple way to discriminate between two well characterized phenotypes. Immature

25   dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor (MR). The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T-cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and

30   costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1 BB).

- 36 -

As noted above, certain preferred embodiments of the present invention employ dendritic cells (DCs), or progenitors thereof, as antigen-presenting cells. Dendritic cells express a number of surface molecules, exemplified herein by DEC-205 and CD11c, which are restricted in expression to DC. It is contemplated, however, that DC may, alternatively,

5   be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are within the scope of the present invention.

Alternative embodiments of the present invention provide *in vivo* methods for introducing a protein-antigen into an APC and/or a DC of a patient, preferably a human

10   patient. Such methods comprise the step of administering to the patient a composition comprising an scFv/antigen complex as disclosed herein above and as exemplified below by the scFv NLDC-85B antigen complex. In related embodiments, one or more polynucleotide encoding an APC and/or DC-specific scFv/antigen may be administered thus utilizing *in vivo* expression of the scFv/antigen coding region.

15   Still further aspects of the present invention provide methods for enhancing, stimulating, suppressing, and/or blocking an immune response in a patient as well as methods for treating a disease in a patient, the methods comprising the steps of: (a) obtaining from the patient a sample comprising an antigen-presenting cell (APC) and/or a dendritic cell (DC); (b) contacting the sample with an scFv/antigen complex under

20   conditions and for such a time as required to allow binding of the scFv/antigen complex to the APC and/or DC; and (c) administering the scFv/antigen APC and/or DC-bound complex to the patient.

Within such methods, the immune response may be a cellular response, such as a T-cell response, or an antibody response. Exemplary cellular responses include a T_h1

25   response, a T_h2 response, and a Cytotoxic T-cell (CTL) response. Exemplary antibody responses include IgM, IgD, IgG_3, IgG_1, IgG_{2b}, IgG_{2a}, IgE, and IgA responses.

Within such methods, the disease may be selected from the group consisting of an infectious disease and cancer. More preferred methods provide that the infectious disease is caused by a virus, a parasite, or a bacterium. Exemplary viral organisms include, but are not

30   limited to, human immunodeficiency virus (HIV), a herpes virus, and an influenza virus.

- 37 -

Exemplary parasitic organisms include, but are not limited to, *Leishmania (e.g., L. major* and *L. donovani)*. Exemplary bacterial organisms include, but are not limited to, *Mycobacteria (e.g., M. tuberculosis* and *M. bovis)*, *Chlamydia (e.g., C. trachomatis* and *C. pneumoniae)*, and *Ehrlichia* (e.g., *E. sennetsu, E. chaffeensis, E. ewingii,* and *E.*

5   *phagocytophila)*. Cancers that may be amenable to treatment with the methods of the present invention include, but are not limited to, soft tissue sarcomas, lymphomas, and cancers of the brain, esophagus, uterine cervix, bone, lung, endometrium, bladder, breast, larynx, colon/rectum, stomach, ovary, pancreas, adrenal gland and prostate.

       Other aspects provide methods for inhibiting, reducing, suppressing and/or blocking

10   the activity of a target antigen on the surface of an antigen-presenting cell (APC) and/or a dendritic cell (DC), the methods comprising the steps of: (a) obtaining a sample comprising and APC and/or a DC; (b) contacting the APC and/or DC with an scFv capable of specifically binding to the target antigen the surface of the APC and/or DC under conditions and for such a time as required to permit binding of the scFv to the APC and/or DC, wherein

15   binding of the scFv to the APC and/or DC blocks the activity of the target antigen.

       By any of the methods disclosed herein, the scFv may bind to a molecule, including a carbohydrate molecule or a protein molecule, on the surface of the APC and/or DC. Preferred surface protein molecules include, but are not limited to, the mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1, B7-2, CD40, CD11c, DEC-205, a Toll-like

20   receptor (TLR), and the Fcγ receptor (FcγR).

       Within certain methods, the scFv may be complexed to an antigen wherein scFv/antigen complexes are achieved by chemical crosslinking or wherein scFv/antigen complexes are scFv/antigen fusion proteins. Alternatively, the scFv may be complexed with a liposome, a vesicle, a micelle and/or a microsphere.

25   The scFv and scFv/antigen complexes of the invention may be administered prophylactically or therapeutically to an individual already suffering from the disease. In either case, the efficacy of the scFv and/or scFv/antigen complex will depend upon the modulation of the patient's immune response. For example, scFv administered alone may be effective in blocking the target molecule on the APC and/or DC and, consequently, may

30   reduce the intensity of an immune response. On the other hand, scFv/antigen complexes

- 38 -

may stimulate an antigen-specific immune response, for example, by activating cytokine release from helper T-cells and/or by stimulating cytotoxic T-cells (CTL). In addition, or alternatively, scFv/antigen complexes may also stimulate B-cells to produce antibody including IgM, IgD, IgG_3, IgG_1, IgG_{2b}, IgG_{2a}, IgE, and/or IgA.

ScFv- and/or scFv/antigen-based compositions may be administered to a patient in an amount sufficient to modulate the immune response. An amount adequate to accomplish this is defined as "therapeutically effective dose" or "immunogenically effective dose." Amounts effective for this use will depend, for example, on the precise composition composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization dose (that is for therapeutic or prophylactic administration) from about 0.01 mg to about 50 mg per 70 kilogram patient, more commonly from about 0.5-1 mg to about 10-15 mg per 70 kg of body weight. Boosting dosages are typically from about 0.01 mg to about 50 mg of peptide, more commonly about 0.5-1 mg to about 10-15 mg, using a boosting regimen over weeks to months depending upon the patient's response and condition. A suitable protocol would include injection at time 0, 2, 6, 8, 10 and 14 weeks, followed by booster injections at 24 and 28 weeks. Booster injections can be from one, two, three, four, five or more. Initial and booster injection amounts and timing are determined based on the judgment of the physician and the antigen being administered. In one embodiment, the initial and booster dose is 1.3 mg, 4 mg, or 13 mg, administered via intramuscular injection, with at least one and up to 3 booster injections at 8 week intervals, or at least one and up to 4 booster injections at 6 week intervals.

Within specific methods for stimulating an immune response against a *mycobacterial* antigen, a prime/boost regimen may be employed wherein a first immunization comprises pcDNA3/scFv/ NLDC-85B and pcDNA3/scFv/N418-85B vectors and/or protein followed by a second immunization with *M. bovis* Bacille Calmette Guerin (BCG).

It has been shown that bacterial infection of dendritic cells, in particular *mycobacterial* infection, results in the upregulation of the regulatory cytokine IL-12 and of

- 39 -

inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF) which may contribute to the acquired specific resistance against bacterial infection and may promote the development of delayed-type hypersensitivity (DTH). Demangel *et al., Immunol. and Cell Biol.* 78:318-324 (2000). Accordingly, depending on the particular application

5   contemplated, it may be desirable to employ one or more of these, or other, cytokines in conjunction with the scFv/antigen to improve therapeutic efficacy over that achieved with scFv/antigens alone. Thus, compositions and methods of the present invention may further comprise a cytokine selected from the group consisting of IL-1, IL-4, IL-6, IL-12, IFNγ, GM-CSF, and TNF.   Alternatively or additionally, compositions of the present invention

10   may comprise a lipopolysaccharide (LPS) or other modulator of the DC response to antigen. Treatment regimens may employ one or more cytokine administered separately from administration of the scFv/antigen complex.

- 40 -

The following Examples are offered by way of illustration not limitation.

Example 1

GENERATION OF PLASMID CONSTRUCTS ENCODING ANTI-DEC-205 AND ANTI-CD11C

5   SINGLE-CHAIN VARIABLE REGION FRAGMENTS (SCFVS)

This example discloses the generation of scFv that specifically bind to DEC-205 and
CD11c.

Hybridoma cell lines expressing rat anti-DEC-205 monoclonal antibody NLDC-145

10   (ATCC Accession No. HB-290; Inaba et al., Cellular Immunology 163:148-56 (1995) and
Witmer-Pack et al., Cellular Immunology 163:157-62 (1995)) and the hamster anti-CD11c
monoclonal antibody hybridoma N418 (ATCC Accession No. HB-224; Metlay et al., J. Exp.
Med. 171:1753-1771 (1990)), were cultured in RPMI 1640 supplemented with 5 % fetal
bovine serum (FBS), 50 μM β-mercaptoethanol (BME) and 2 mM Glutamine (Gln).

15   Monoclonal antibodies were purified from culture supernatants by chromatography on a
protein-G column (Pharmacia; Peapack, New Jersey, USA). Sf21 insect cells (Clontech;
Palo Alto, California, USA) were propagated at 27°C in Grace's Medium supplemented with
10 % FBS and 2 mM Gln (Gibco BRL/Life Technologies). Passages 1 and 2 were
performed on Sf21 monolayers, and passage 3 in suspension culture with culture medium

20   supplemented with 1% Pluronic F-68® (BASF Corporation).

Total mRNA was extracted from NLDC-145 and N418 hybridoma cells using
RNAzol (Cinna/Tel-Test, Inc.; Friendswood, Texas, USA), and first strand complementary
DNA (cDNA) synthesized using reverse transcriptase and random hexamers by employing
the Recombinant Phage Antibody system (Pharmacia; Uppsala, Sweden). Heavy and light

25   chain variable regions (V$_H$ and V$_L$) of the NLDC-145 and N418 rat immunoglobulin genes
were PCR amplified from these cDNAs using a collection of primers originally designed for
murine antibodies (Recombinant Phage Antibody System, Pharmacia). The nucleotide and
amino acid sequences of scFv NLDC145 are presented herein in SEQ ID NOs: 4 and 7,
respectively. The nucleotide and amino acid sequences of scFv N418 are presented herein

30   in SEQ ID NOs: 1 and 2, respectively.

- 41 -

Polynucleotides encoding the V$_H$ and V$_L$ regions were operably linked to a polynucleotide encoding a peptide linker having the amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser such that the V$_H$ coding region was ligated 5' to the linker encoding polynucleotide and the V$_L$ coding region was ligated 3' to the
5   linker encoding polynucleotide to yield a 750 bp long polynucleotide fragment encoding an NLDC-145-derived anti-DEC-205 scFv having the arrangement V$_H$-linker-V$_L$.

For immunogenicity experiments, the scFvs were fused to the gene encoding the mycobacterial antigen Ag85B. Kamath et al., Clinical and Experimental Immunology 120:476-482 (2000). To allow expression of the product, the N-terminal end of the scFv
10   was fused to HBM sequence. Reavy et al., Protein Expression and Purification 18:221-228 (2000). Moreover, in order to facilitate its detection and purification, the C-terminal end of the scFv was fused to the DYKDDDDK peptide (FLAG) and the resulting product subcloned in pCDNA3 expression vector (Invitrogen).

The genes encoding the variable regions of the heavy (V$_H$) and light (V$_L$) chains of
15   NLDC-145 and N418 were amplified by PCR. Deduced amino acid sequence of the resulting products displayed the typical architecture of immunoglobulin variable domains (Figure 3A). Each V$_H$ fragment was then bound to its V$_L$ partner by use of a spacer encoding a 15 amino-acid flexible linker, yielding scFv constructs ScNLDC and ScN418.

20   Example 2

EXPRESSION AND PURIFICATION OF ANTI-DEC-205 SCFV NLDC-145 AND ANTI-CD11C SCFV N418

This example discloses the expression and purification of the anti-DEC-205 scFv
25   designated scFv NLDC-145 and the anti-CD11c scFv designated scFv N418.

To examine the binding specificities of ScNLDC and ScN418, these NLDC-145-derived anti-DEC-205 scFv and N418-derived anti-CD11c scFv were expressed in a baculovirus expression system. For this purpose, the N-terminus of ScNLDC and ScN418 were fused to the honeybee melittin leader sequence (HBM; Reavy *et al.*, *Protein*
30   *Expression and Purification* 18:221-228 (2000)), and their C-terminal end to a FLAG peptide and a hexahistidine tail. Figure 3B shows that cells infected with ScNLDC

- 42 -

recombinant baculovirus released a 30 kD protein, identified as ScNLDC by Western blot
analysis using anti-FLAG and anti-hexahistidine antibodies. Comparably to ScNLDC,
ScN418 was successfully produced and secreted by insect cells infected with ScN418
recombinant baculovirus (not shown), allowing us to purify both scFv products from insect
5   cell culture supernatants by FLAG affinity-based chromatography.

The polynucleotide encoding the NLDC-145-derived anti-DEC-205 scFv described
in Example 1 was cloned into the baculovirus expression vector pBACPak8 (Clontech; Palo
Alto, California) to generate the plasmid vector designated pBCV/NLDC-145 presented
herein as Figure 1A. Similarly, the polynucleotide encoding the N418-derived anti-CD11c
10   (SEQ ID NO: 3) scFv was cloned into pBACPak8 to generate the plasmid vector designated
pBCV/N418.

For expression in insect cells, the NLDC-145 scFv and the N418 scFv
polynucleotides were each ligated in-frame with a polynucleotide encoding a FLAG peptide
(DYKDDDDK) and a poly-Histidine tail within the transfer vector pBacPAK8 (Clontech:
15   Palo Alto, California, USA). Plasmid and baculovirus DNA were co-transfected into Sf21
cells in the presence of Lipofectin (Gibco BRL/Life Technologies) and recombinant viruses
amplified according to the protocol recommended by the manufacturer. The HBM-leaded
recombinant protein was secreted by the virus particles and recovered from infected Sf21
culture medium. The NLDC-145 scFv and the N418 scFv polypeptides were adsorbed onto
20   an anti-FLAG M2 affinity column as described by the manufacturer (Sigma-AldrichCorp.;
St. Louis, MO, USA) and eluted with 0.1M glycine, with immediate neutralization by Tris
0.1 M pH 8.0. Purified NLDC-145 scFv and N418 scFv were analyzed by SDS-PAGE on a
12% acrylamide gel followed by silver staining, and by Western-blot using the anti-FLAG
M2 and the anti-polyhistidine His1 antibodies (both from Sigma).

25   For expression in insect cells, scFv constructs tagged with a FLAG peptide and a
hexahistidine tail were subcloned in the transfer vector pBacPAK8 (Clontech). Plasmid and
baculovirus DNA were co-transfected into Sf21 cells in the presence of Lipofectin (Gibco
BRL/Life Technologies), and recombinant viruses amplified according to the manufacturer's
protocol. The HBM-leaded recombinant protein was secreted by the virus particles and
30   could be recovered from infected Sf21 culture medium. Passage 3 supernatant was purified

- 43 -

by affinity chromatography on an anti-FLAG M2 gel, as described by the manufacturer (Sigma). Acid elution was performed with 0.1M glycine, with immediate neutralization by Tris 0.1M pH 8.0. Elution fractions were analyzed by SDS-PAGE on a 12% acrylamide gel followed by silver staining, and by Western-blot using the anti-FLAG M2 and the anti-

5   polyhistidine His1 antibodies (both from Sigma).

Example 3

BINDING OF THE ANTI-DEC-205 scFv NLDC-145 TO LANGERHANS CELLS

10   This example demonstrates that scFv NLDC-145 is capable of specifically binding to murine dendritic cells (Langerhans cells).

         Epidermal sheets of mouse ears were prepared as described. Halliday et al., Immunology 77(1):13-8 (1992). Epidermis were incubated with scNLDC (20µg/ml), purified as described in Example 2, for 72 h at 4°C, followed by 10µg/ml M2 or a 1:100

15   dilution of His1 for 16 h at 4°C, biotinylated goat anti-mouse antibody (1:200) 16 h at 4°C, and streptavidin-conjugated alkaline phosphatase (1:200) for 2 h at RT. Control epidermis were stained with equivalent binding site molar concentration (50µg/ml) of purified parental NLDC-145 monoclonal antibody, followed by biotinylated goat anti-rat antibody (1:200) for 16 h at 4°C, and streptavidin-conjugated alkaline phosphatase (1:200) for 2 h at RT.

20   ScN418 binding to CD11c was assessed on FSDCs, by incubation of fixed cells with purified ScN418 (10µg/ml) for 1h at RT, followed by 10µg/ml M2 or a 1:100 dilution of His1 for for 1h at RT, horseradish peroxidase-conjugated anti mouse antibody for 1 h at RT.

         Control cells were stained with equivalent binding site molar concentration (25µg/ml) of biotinylated N418 antibody, followed by streptavidin-conjugated horseradish

25   peroxidase for 1 h at RT. Controls included epidermal sheets incubated with secondary reagents only. Each incubation step was performed in DMEM + 10% FCS and was followed by three 2 h washes in PBS with gentle agitation. After final washing, epidermis were stained with a fuchsin-based alkaline phosphatase substrate for 20 min, and mounted on glass microscope slides in Histomount. Epidermal sheets of mouse ears stained with

30   NLDC-145 parental monoclonal antibody or with purified scFv NLDC-145, as detected via the C-terminal poly-His tail or FLAG peptide, were compared. The scFv NLDC-145 bound

- 44 -

to the subcutaneous dendritic cells with the same specificity as did the parental monoclonal antibody.

As mouse epidermal DC (or Langerhans cells) display a distinctive DEC-205^{high} phenotype (Anjuere *et al.*, *Blood* 93:590-8 (1999)), we used epidermal sheets of mouse ears

5   to examine the ability of ScNLDC to bind its target receptor. Staining of mouse epidermis with 50μg/ml purified NLDC-145 antibody revealed the characteristic network formed by Langerhans cells (Fig. 4A). When mouse epidermal tissues were incubated with 20μg/ml ScNLDC (an antigen binding site concentration equivalent to 50μg/ml NLDC-145), an equivalent phenotype was detected, using either anti-hexahistidine or anti-FLAG detection

10 antibodies (Figures 4B and 4D). No signal was observed in the absence of ScNLDC (Figure 4C), demonstrating that ScNLDC retains the specificity of the parental antibody.

The ability of ScN418 to bind CD11c was similarly investigated on the dendritic cell line FSDC. Girolomoni *et al.*, *European J. Immunol.* 25:2163-2169 (1995). Staining of FSDCs was equivalent using either 10 μg/ml N418 monoclonal antibody or an equivalent

15 concentration of ScN418 (Figures 5A, 5B, and 5D). In contrast, no staining was detected in the absence of the scFv (Figure 5C), or when the cells were incubated with N418 prior to ScN418 (not shown). Therefore, ScN418 binds CD11c with the same specificity as N418.

Example 4

20 GENERATION OF A PLASMID CONSTRUCT ENCODING AN ANTI-DEC-205 SINGLE-CHAIN FV- *M.*
TUBERCULOSIS ANTIGEN 85B FUSION PROTEIN

This example discloses the generation of a scFv/antigen complex comprising scFv NLDC-145, described above, fused to the protein-antigen 85B from *mycobacterium*

25 *tuberculosis*.

A polynucleotide encoding scFv NLDC-145 was ligated in-frame with a polynucleotide encoding the *mycobacterial* protein-antigen 85B. Kamath *et al.*, *Infection and Immunity* 67(4):1702-1707 (1999). To facilitate expression of scFv/antigen complex, a polynucleotide encoding the honeybee melittin signal peptide (HBM) was ligated 5' to and

30 in-frame with the polynucleotide encoding scFv NLDC-145. Tessier *et al.*, *Gene* 98(2):177-83 (1991). A polynucleotide encoding a linker polypeptide (SEQ ID NO: 11) was ligated 3'

- 45 -

to and in-frame with the polynucleotide encoding scFv NLDC-145 and a polynucleotide

encoding the *M. tuberculosis* protein-antigen 85B (SEQ ID NO: 13) was ligated 3' to and in-

frame with the polynucleotide encoding the linker polypeptide. This fusion polynucleotide

construct was cloned into the pcDNA3 plasmid vector (Invitrogen, Carlsbad, California) to

5   generate pcDNA3-NLDC-85B. (Figure 1B). The nucleotide sequence of scFv NLDC-145-

85B is presented in SEQ ID NO: 8.

COS cells were transfected with pcDNA3-NLDC-85B and a control plasmid,

pcDNA3-85B, that expresses the 85B antigen alone. Expression of the anti-DEC-205 scFv

NLDC-85B fusion protein and 85B protein-antigen were readily detected in extracts of the

10   COS cells by standard immunoblotting methodology.

Example 5

TARGETING OF SCFV NLDC-85B TO DENDRITIC CELLS STIMULATES
85B-SPECIFIC T-CELL RESPONSES

15

This Example demonstrates that scFv NLDC-85B specifically binds dendritic cells

and facilitates a T-cell response against the *M. tuberculosis* 85B protein-antigen.

The immune response facilitated by plasmid vectors pcDNA3-NLDC-85B and

pcDNA3-85B were compared in C57BL/6 mice. C57Bl/6 female mice were supplied as

20   specific-pathogen-free mice by the Animal Resource Centre (Perth, Australia) and were

maintained under specific-pathogen-free conditions. Mice were immunized at 8 weeks of

age. For intramuscular injections, 50µg of plasmid was injected into the tibialis anterior

muscle of each hindleg. For intradermal injections, the same quantity of DNA was

delivered in the dermis of each ear. Control mice were immunized with pcDNA3, or with

25   the pcDNA3 vector expressing Ag85B in the absence of scFv NLDC-145. Mice were

immunized either one or two times at 2-week intervals, and sacrified 4 weeks after the last

injection for immunogenicity studies.

To test the impact of antigen targeting on dendritic cells, DNA vaccine vectors

encoding fusion proteins between scFvs and a model mycobacterial antigen (Ag85B) were

30   designed. Kamath *et al., supra.* For product detection purposes, the plasmid vectors

(pScNLDC-Ag85B and pScN418-Ag85B) contained a FLAG sequence linked to the 3' of

- 46 -

the Ag85B gene (Figure 2). Western blot analysis using anti-Ag85B and anti-FLAG detection antibodies identified a product expressed by COS7 cells transfected with pScNLDC-Ag85B or pScN418-Ag85B. Introduction of the targeting sequence had no impact on Ag85B expression, as COS7 cells transfected with pScNLDC-Ag85B, pScN418-

5   Ag85B or with a plasmid encoding the Ag85B gene without scFv (pAg85B) expressed comparable amounts of Ag85B (not shown). These DNA vectors were then used to immunize mice via the intramuscular route, and compared for their ability to generate antibodies and IFN-γ secreting T cells specific of Ag85B.

Spleens from the sacrificed mice were harvested, splenocytes isolated and cultured in
10   the presence of *M. tuberculosis* antigen 85 to measure Interferon-γ (IFN-γ) release and to quantify the number of IFN-γ-secreting T-cells. Mice immunized with pcDNA3-NLDC-85 induced a stronger IFN-γ secreting T-cell response against antigen 85B than mice immunized with antigen 85B alone, with a more than two-fold increase the number of specific IFN-γ secreting T-cells. (Figure 6). The generation of Th1-type T cells specific of
15   Ag85B was also enhanced, as evidenced by the increased frequency of antigen specific IFN-γ secreting cells in mice immunized by one injection of pScNLDC-Ag85B.

Ag85B specific antibodies in sera were assayed for by ELISA using purified protein-antigen 85B as described in Palendira *et al.*, *Infection and Immunity* 70(4):1949-1956 (2002). Lymphocyte proliferation assays, ELISA and ELISPOT for IFN-γ production were
20   conducted as previously described. Kamath *et al.*, *Infection and Immunity* 67(4):1702-1707 (1999). The effects on antibody production were also tested after 1 and 2 injections to the DNA vaccines. The pcDNA3-NLDC-85B vector induced a small but significant increase in IgG antibody titer specific for antigen 85B. (Figure 7). Two weeks after a single dose of DNA vaccine, the titer of anti-Ag85B IgG was significantly higher in the group immunized
25   with pScNLDC-Ag85B, as compared to the group vaccinated with pAg85B, demonstrating that Ag85B targeting to DEC-205 enhanced the production of specific antibodies. In contrast, Ag85B fusion to scFv N418 did not result in enhanced immunogenicity (not shown).

- 47 -

These data demonstrated that targeting of the DC-specific receptor DEC-205 with an anti-DEC-205 scFv-NLDC-85B protein-antigen complex enhanced the cellular immune response to antigen 85B.

5   Example 6

In vivo Administration of Polynucleotides Encoding
scFv/NLDC145-85B and scFv/N418-85B

Polynucleotides encoding scFv/NLDC145-85B and scFv/N418-85B were
10   administered *in vivo* to enhance an immune response against tuberculosis. For example, the scFv NLDC-85B DNA vaccine construct expressing the anti-DEC-205 svFv fused to the *M. tuberculosis* Antigen 85B (100 μg) was used to immunize C57BL6 mice (n=5) three times by the intramuscular route at two weekly intervals. Other groups of mice received pCDNA-85B expressing the mature Antigen 85B protein alone (100 μg) or the control vector
15   pCDNA3 (100 μg) by the intramuscular route or the currently used live vaccine *M. bovis* BCG (Pasteur strain; 5x10⁴) once by the subcutaneous route. The mice were rested 6 weeks after the last DNA immunization or 12 weeks after the BCG vaccine. The mice were then infected, by the aerosol route, with 100 cfu of virulent *Mycobacterium tuberculosis* H37Rv using a Middlebrook aerosol infection apparatus (Glas-Col, Terre Haute, IN). The mice
20   were sacrificed 4 weeks later and the number of organisms in the lung and spleen enumerated by culture on OADC supplemented Middlebrook 7H11 agar (Difco Laboratories).

Mice immunised with the scFv NLDC-85B DNA vaccine had significantly less *M. tuberculosis* organisms in the lung (p<0.05) and in the spleen (p<0.05) than the recipients of
25   the non-targeted DNA-85B (Figure 8). There was no significant difference between the protective efficacy of the targeted scFv NLDC-85B DNA vaccine and the live vaccine BCG.

This indicates that targeting the mycobacterial protein to Dendritic Cells (DCs) with the scFv specific for the surface protein DEC-205 has increased the protective effect of DNA immunisation at the primary site of tuberculosis infection in the lung. The increase in
30   the protective effect in the spleen indicates that this vaccine strategy has also reduced dissemination of *M, tuberculosis* organisms from the site of infection in the lungs to other

- 48 -

organs. In previous studies with anti-tuberculosis subunit vaccines, immunisation with a single antigen, either as DNA or protein, had limited effect on blocking spread from the lung.

5   Example 7
PRIME/BOOST IMMUNIZATION REGIMEN EMPLOYING *A* PCDNA3 NLDC-85B OR PCDNA3 N418-85B PRIME FOLLOWED BY A BCG BOOST

Prior studies demonstrated that a prime/boost combination of DNA immunization
10   prior to BCG immunization dramatically increases the effectiveness of the BCG vaccine. Accordingly, in this Example, mice are immunized with pcDNA3/scFv/ NLDC-85B and pcDNA3/scFv/N418-85B vectors followed by BCG in a prime/boost regimen. Mice are immunized by intramuscular injection of 2 doses, 100 µg for each vector, or pcDNA3-85B or the negative control pcDNA3 vector. Two weeks after the second injection the same
15   mice are immunized subcutaneously with 5×10^4 BCG organisms. Six weeks later the protective effect is assessed by aerosol challenge with *M. tuberculosis* H37RB, as presented in Example 6, and the protective effect assayed 4 weeks after challenge. This prime/boost strategy using the pcDNA3/scFv/NLDC-85B and pcDNA3/scFv/N418-85B will also be testing in the guinea pig model of aerosol *M. tuberculosis* infection.

20
Example 8

TARGETING OF SCFV TUMOR ANTIGENS TO DENDRITIC CELLS STIMULATES
TUMOR ANTIGEN-SPECIFIC T-CELL RESPONSES

25   As presented in Example 5, a polynucleotide encoding scFv NLDC-85B increased T-cell and antibody responses to *Mycobacterial* antigen 85B. Similarly, this approach may be employed to stimulate a T-cell response against tumor antigens thereby increasing the clearance of immunologically sensitive cancers. For example, a polynucleotide encoding a scFv ovalbumin fusion protein is prepared and mice immunized with this polynucleotide,
30   and a control polynucleotide. Following immunization, mice are challenged with tumors bearing the ovalbumin gene including the EL4 thymoma to demonstrate the effectiveness of the scFv ovalbumin fusion protein in reducing cell growth in El4 thymoma or other cells.

- 49 -

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or

5   indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

- 50 -

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1.   A single-chain variable region fragment (scFv), comprising a heavy chain variable region (V_H) operably linked to a light chain variable region (V_L) wherein said scFv is

5   capable of specifically binding to a molecule on the surface of an antigen-presenting cell (APC).

2.   The scFv of claim 1 wherein said APC is a dendritic cell (DC).

10   3.   The scFv of claim 1 wherein said molecule is selected from the group consisting of the mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1 (CD80), B7-2 (CD86), CD40, CD11c, DEC-205, a Toll-like receptor (TLR), and the Fcγ receptor (FcγR).

4.   The scFv of claim 3 wherein said molecule is DEC-205. .

15

5.   The scFv of claim 3 wherein said molecule is CD11c.

6.   The scFv of claim 1 wherein said scFv further comprises a polypeptide linker operably linked between said V_H region and said V_L region.

20

7.   The scFv of claim 1 wherein said scFv further comprises an affinity tag.

8.   The scFv of claim 7 wherein said affinity tag comprises one or more hexahistidine.

25   9.   The scFv of claim 1 wherein said V_H region and said V_L region are each at least 70% identical to the V_H and V_L regions of monoclonal antibody NLDC-145 disclosed herein in SEQ ID NOs: 5 and 6, respectively.

30   10.   The scFv of claim 1 wherein said V_H region and said V_L region are each at least 90% identical to the V_H and V_L regions of monoclonal antibody NLDC-145 disclosed herein in SEQ ID NOs: 5 and 6, respectively.

- 51 -

11.   An scFv/antigen complex, comprising an scFv of any one of claims 1-10 complexed with an antigen.

5   12.   The scFv/antigen complex of claim 11 wherein said complex comprises a chemical crosslink between said scFv with said antigen.

13.   The scFv/antigen complex of claim 11 wherein said complex comprises a fusion protein comprising said scFv and said antigen.

10

14.   The scFv/antigen complex of claim 11 wherein said scFv further comprises an affinity tag.

15.   The scFv/antigen complex of claim 14 wherein said affinity tag comprises one or

15   more hexahistidine.

16.   The scFv/antigen complex of claim 11 wherein said complex further comprises a lipid.

20   17.   The scFv/antigen complex of claim 16 wherein said lipid is a metal-chelating lipid.

18.   The scFv/antigen complex of claim 17 wherein said metal-chelating lipid is nitrilotriacetic acid ditetradecylamine.

25   19.   The scFv/antigen complex of claim 11 wherein said antigen is from a bacterium selected from the group consisting of *Mycobacterium, Chlamydia,* and *Ehrlichia.*

20.   The scFv/antigen complex of claim 19 wherein said antigen is a *Mycobacterial* antigen, or fragment, derivative, or variant thereof, selected from the group consisting of

30   85B, MPT64, and ESAT-6 as presented herein in SEQ ID NO:14, SEQ ID NO: 16, and SEQ ID NO: 18, respectively.

- 52 -

21.   The scFv/antigen complex of claim 20 wherein said antigen is a variant of said *Mycobacterial* antigen 85B wherein said variant is at least about 70% identical to the sequence presented herein in SEQ ID NO: 14.

22.   The scFv/antigen complex of claim 21 wherein said antigen is a variant of said *Mycobacterial* antigen 85B wherein said variant is at least about 90% identical to the sequence presented herein in SEQ ID NO: 14.

23.   The scFv/antigen complex of claim 22 wherein said scFv/antigen complex is the scFv NLDC-145-85B presented herein in SEQ ID NO: 8, or fragment, derivative, or variant thereof.

24.   The scFv/antigen complex of claim 23 wherein said scFv/antigen complex is at least about 70% identical to the scFv NLDC-145-85B presented herein in SEQ ID NO: 8.

25.   The scFv/antigen complex of claim 23 wherein said scFv/antigen complex is at least about 90% identical to the scFv NLDC-145-85B presented herein in SEQ ID NO: 8.

26.   The scFv/antigen complex of claim 11 further comprising a cytokine selected from the group consisting of IL-12, IL-6, IL-4, IL-1, IFNγ, GM-CSF, and TNF.

27.   The scFv/antigen complex of claim 11 further comprising an inducer of a DC response to said antigen wherein said inducer is selected from the group consisting of a lipopolysaccharide (LPS) or other cell wall component, a non-methylated CpG motif, and a double-stranded RNA.

28.   A fusion protein comprising an antigen-presenting cell (APC) binding protein and an antigen wherein said fusion protein is capable of specifically binding to a molecule on the surface of an APC and inducing an antigen specific T-cell response.

- 53 -

29.   The fusion protein of claim 28 wherein said molecule on the surface of said APC is selected from the group consisting of the mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1 (CD80), B7-2 (CD86), CD40, CD11c, DEC-205, a Toll-like receptor (TLR), and the Fcγ receptor (FcγR).

5

30.   The fusion protein of claim 28 wherein said molecule on the surface of said APC is DEC-205.

31.   The fusion protein of claim 28 wherein said molecule on the surface of said APC is

10   CD11c.

32.   The fusion protein of claim 28 wherein said antigen is a *Mycobacterial* antigen, or fragment, derivative, or variant thereof, selected from the group consisting of 85B, MPT64, and ESAT-6 as presented herein in SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18,

15   respectively.

33.   The fusion protein of claim 28 wherein said antigen is a variant of said *Mycobacterial* antigen 85B wherein said variant is at least about 70% identical to the sequence presented herein in SEQ ID NO: 14.

20

34.   The fusion protein of claim 28 wherein said antigen is a variant of said *Mycobacterial* antigen 85B wherein said variant is at least about 90% identical to the sequence presented herein in SEQ ID NO: 14.

25   35.   The fusion protein of claim 28 wherein said antigen comprises said *Mycobacterial* antigen 85B presented herein in SEQ ID NO: 14.

36.   A polynucleotide for expressing an scFv/antigen complex, comprising a first polynucleotide operably linked to a second polynucleotide wherein said first polynucleotide

30   encodes an scFv of any one of claims 1-15 and wherein said second polynucleotide encodes an antigen.

- 54 -

37.   The polynucleotide of claim 36 wherein said antigen is a *Mycobacterial* antigen, or fragment, derivative, or variant thereof, selected from the group consisting of 85B, MPT64, and ESAT-6 as presented herein in SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18, respectively.

38.   The polynucleotide of claim 37 wherein said antigen is a variant of said *Mycobacterial* antigen 85B wherein said variant is at least about 70% identical to the sequence presented herein in SEQ ID NO: 14.

39.   The polynucleotide of claim 37 wherein said antigen is a variant of said *Mycobacterial* antigen 85B wherein said variant is at least about 90% identical to the sequence presented herein in SEQ ID NO: 14.

40.   The polynucleotide of claim 39 wherein said scFv/antigen complex is at least about 70% identical to the scFv NLDC-145-85B presented herein in SEQ ID NO: 8.

41.   The polynucleotide of claim 39 wherein said scFv/antigen complex is at least about 90% identical to the scFv NLDC-145-85B presented herein in SEQ ID NO: 8.

42.   A polynucleotide comprising a polynucleotide that encodes a fusion protein of any one of claims 28-35.

43.   A vector comprising a polynucleotide of any one of claims 36-42 operably linked to a transcriptional promoter.

44.   A method for introducing an antigen into an antigen-presenting cell (APC) and/or a dendritic cell (DC), said method comprising the steps of:

        (a)   isolating from a patient a sample comprising an APC and/or a DC; and

        (b)   contacting said APC and/or said DC with the scFv/antigen complex of claim 16,

- 55 -

wherein said scFv/antigen complex is in contact with said APC and/or said DC under conditions and for such time as required to permit said antigen to enter said APC and/or said DC.

45.   A method for introducing an antigen into an antigen-presenting cell (APC) and/or a dendritic cell (DC) of a patient, said method comprising the step of administering to said patient a composition comprising the scFv/antigen complex of claim 16.

46.   A method for introducing an antigen into an antigen-presenting cell (APC) and/or a dendritic cell (DC) of a patient, said method comprising the step of administering to said patient a composition comprising a polynucleotide of claim 36.

47.   A method for inducing an immune response in a patient, said method comprising the steps of:

(a)   obtaining from said patient a sample comprising an antigen-presenting cell (APC) and/or a dendritic cell (DC);

(b)   contacting said sample with the scFv/antigen complex of claim 16 under conditions and for such a time as required to allow binding of said scFv fragment antigen complex to said APC and/or said DC; and

(c)   administering said scFv/antigen APC and/or DC-bound complex to said patient.

48.   A method of blocking, or substantially reducing, the activity of a target molecule on the surface of an antigen-presenting cell (APC) and/or a dendritic cell (DC), said method comprising the steps of:

(a)   isolating from said patient a sample comprising an APC and/or a DC; and

(b)   contacting said APC and/or said DC with the scFv of claim 1 under conditions and for such time as required to permit said binding of said scFv to said target antigen,
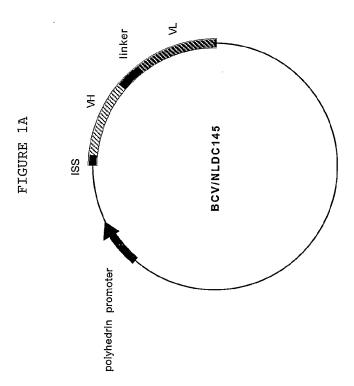
wherein binding of said scFv to said target molecule blocks, or substantially reduces, the activity of said target molecule.
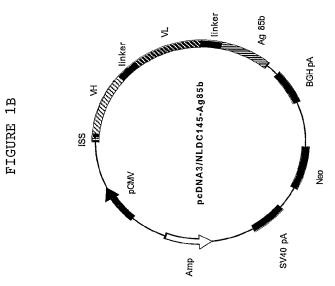
- 56 -

49.   The method of claim 48 wherein said target molecule is a receptor protein selected from the group consisting of the mannose receptor (MR), chemokine receptor 1 (CCR1), B7-1 (CD80), B7-2 (CD86), CD40, CD11c, DEC-205, a Toll-like receptor (TLR), and the Fcγ receptor (FcγR).

50.   A method of preventing and/or treating a disease in a patient comprising the steps of:

(a) obtaining from said patient a sample comprising an antigen-presenting cell (APC) and/or a dendritic cell (DC);

(b) contacting the sample with an scFv/antigen complex under conditions and for such a time as required to allow binding of the scFv/antigen complex to the APC and/or DC; and

(c) administering the scFv/antigen APC and/or DC-bound complex to the patient.

51.   A method according to claim 50 wherein the disease is selected from the group consisting of: an infectious disease, an autoimmune disease and a cancer.

52.   A method according to claim 51 wherein the infectious disease is caused by an organism selected from the group consisting of: *Leishmania, Mycobacteria, Chlamydia* and *Ehrlichia* .

53.   A method according to claim 51 wherein the cancer is selected from the group consisting of: soft tissue sarcomas, lymphomas, and cancers of the brain, esophagus, uterine cervix, bone, lung, endometrium, bladder, breast, larynx, colon/rectum, stomach, ovary, pancreas, adrenal gland and prostate.
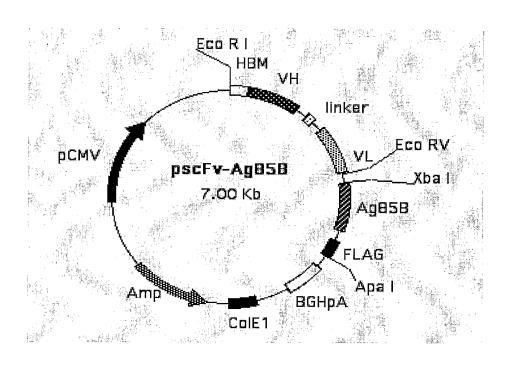
FIGURE 1A

FIGURE 1B

Figure 2
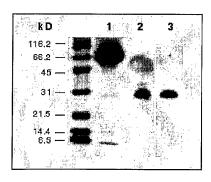
Figure 3

A

```
                      CDR1
NLDC VH    EVKLQQSGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIG
N418 VH    QVKLQESGGGLVQPGRSLKLSCAASGFTFSNSGMHWIRQSPTKGLEWVA
              CDR2
           WIFPGEGSTEYNEKFKGRATLSVDKSSSTAYMELTRLTSEDSAVYFCAR-
           SISPSGGTTYYRDSVKGRFTFSRDNAKSTLYLQMDSLRSEDTATYYSATD
              CDR3
           GDYYRRYFDLWGQGTTVTVSS
           SGHGYTYFDYWGQGTTVTVSS
                      CDR1
NLDC VL    DIQMTQSPSFLSTSLGNSITITCHASQNIKGWLAWYQQKSGNAPQLLIY
N418 VL    DIQMTQSPSSLPASLGDRVTIHCQASQDISNYLTWYQQKPGKAPKLLIY
              CDR2                        CDR3
           KASSLQSGVPSRFSGSGSGTDYIFTISNLQPEDIATYYCQHYQSFPWTFGGGTKLEIKRAA
           ETNKLADGVPSRFSGSGSGRDYSFTISSLESEDVGSYYCQHYYDYPRTFGPGTKLEIKRAA
```
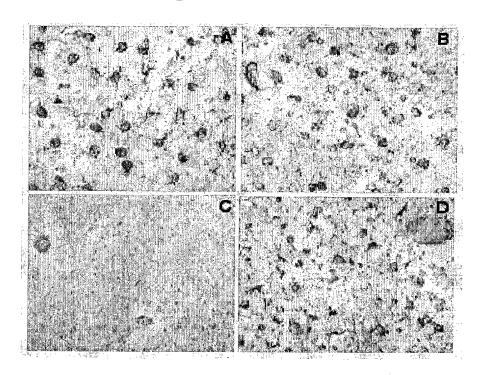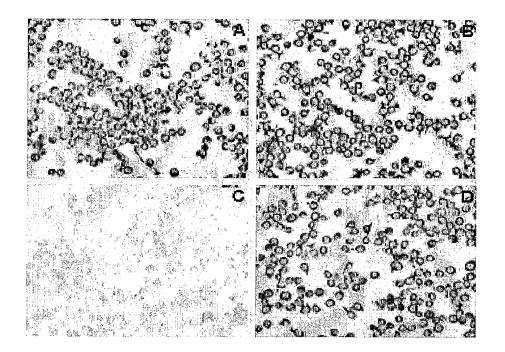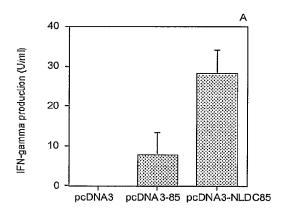
B

Figure 4

Figure 5

Figure 6
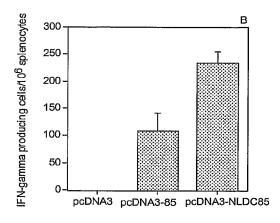
Figure 7
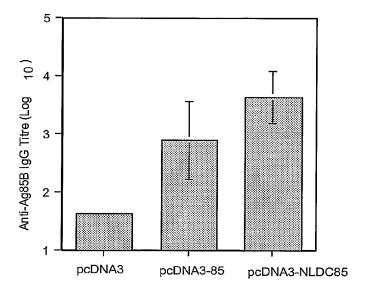
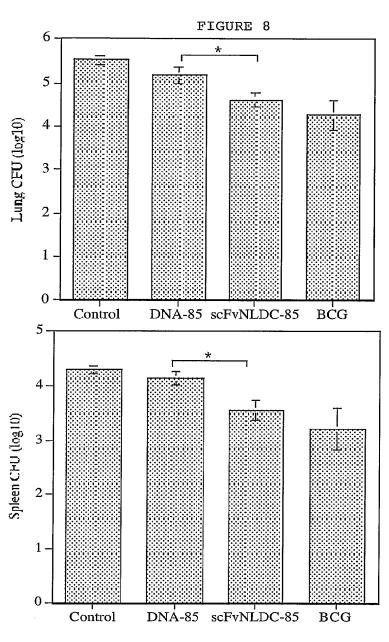FIGURE 8

* p< 0.05

40735WOP00SEQLIST.TXT
SEQUENCE LISTING

<110> Centenary Institute Cancer Medicine & Cell Biology

<120> Compositions and Methods for Targeting
      Antigen-Presenting Cells With Antibody Single-Chain Variable
      Region Fragments

<130> 40735WOP00

<160> 18

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 729
<212> DNA
<213> Artificial sequence

<220>
<223> Nucleotide sequence encoding fusion protein

<400> 1
caggtgaaac tgcaggagtc aggggggcggc ttggtgcagc ctggaaggtc cctgaaactc   60
tcctgtgcag cctcaggatt cactttcagt aactctggca tgcactggat ccgccagtct   120
ccaacgaagg gtctggagtg ggtcgcatcc attagtccta gtggtggtac cacttactat   180
cgagactccg tgaagggccg attcactttc tccagggata atgcaaaaag caccctatat   240
ctgcaaatgg acagtctgag gtctgaggac acggccactt attactgcaa cagatcgggg   300
cacgggtata cctactttga ttactggggc caagggacca cggtcaccgt ctcctcaggt   360
ggaggtggtt caggcggagg tggatccgga ggcggtggat cggacatcca gatgactcag   420
tctccatcat cactgcctgc ctccctggga gacagagtca ctatccattg tcaggccagt   480
caggacatta gcaattattt aacctggtac cagcagaaac agggaaagc tcctaagctc   540
ctgatctatg aaacaaataa attggcagat ggagtcccat caaggttcag tggcagtggt   600
tctgggagag attattcttt cactatcagc agcctggaat ctgaagatgt tggatcttat   660
tactgtcaac attattatga ctatcctcgg acgttcggac ctgggaccaa gctggaaata   720
aaacgggcg   729
                729

<210> 2
<211> 243
<212> PRT
<213> Artificial sequence

<220>
<223> Fusion protein

<400> 2
Gln Val Lys Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1   5   10   15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
            20   25   30
Gly Met His Trp Ile Arg Gln Ser Pro Thr Lys Gly Leu Glu Trp Val
        35   40   45
Ala Ser Ile Ser Pro Ser Gly Gly Thr Thr Tyr Tyr Arg Asp Ser Val
    50   55   60
Lys Gly Arg Phe Thr Phe Ser Arg Asp Asn Ala Lys Ser Thr Leu Tyr
65   70   75   80

<pre>
                              40735WOP00SEQLIST.TXT
Leu Gln Met Asp Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
            85   90   95
Asn Arg Ser Gly His Gly Tyr Thr Tyr Phe Asp Tyr Trp Gly Gln Gly
            100   105   110
Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly Gly Gly
            115   120   125
Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
    130   135   140
Leu Pro Ala Ser Leu Gly Asp Arg Val Thr Ile His Cys Gln Ala Ser
145   150   155   160
Gln Asp Ile Ser Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys
                165   170   175
Ala Pro Lys Leu Leu Ile Tyr Glu Thr Asn Lys Leu Ala Asp Gly Val
            180   185   190
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Thr
            195   200   205
Ile Ser Ser Leu Glu Ser Glu Asp Val Gly Ser Tyr Tyr Cys Gln His
    210   215   220
Tyr Tyr Asp Tyr Pro Arg Thr Phe Gly Pro Gly Thr Lys Leu Glu Ile
225   230   235   240
Lys Arg Ala

<210> 3
<211> 1623
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence encoding fusion protein

<400> 3
caggtgaaac tgcaggagtc aggggggcggc ttggtgcagc ctggaaggtc cctgaaactc   60
tcctgtgcag cctcaggatt cactttcagt aactctggca tgcactggat ccgccagtct   120
ccaacgaagg gtctggagtg ggtcgcatcc attagtccta gtggtggtac cacttactat   180
cgagactccg tgaagggccg attcactttc tccagggata atgcaaaaag caccctatat   240
ctgcaaatgg acagtctgag gtctgaggac acggccactt attactgcaa cagatcgggg   300
cacgggtata cctactttga ttactggggc caagggacca cggtcaccgt ctcctcaggt   360
ggaggtggtt caggcggagg tggatccgga ggcggtggat cggacatcca gatgactcag   420
tctccatcat cactgcctgc ctccctggga gacagagtca ctatccattg tcaggccagt   480
caggacatta gcaattattt aacctggtac cagcagaaac agggaaagc tcctaagctc   540
ctgatctatg aaacaaataa attggcagat ggagtcccat caaggttcag tggcagtggt   600
tctgggagag attattcttt cactatcagc agcctggaat ctgaagatgt ggatcttat   660
tactgtcaac attattatga ctatcctcgg acgttcggac ctgggaccaa gctggaaata   720
aaacggggat atccatcaca ctggcggccg ctcgagcatg catctagatt ctcccggccg   780
gggctgccgg tcgagtacct gcaggtgccg tcgccgtcga tgggccgcga catcaaggtt   840
cagttccaga gcggtgggaa caactcacct gcggtttatc tgctcgacgg cctgcgcgcc   900
caagacgact acaacggctg ggatatcaac accccggcgt tcgagtggta ctaccagtcg   960
ggactgtcga tagtcatgcc ggtcggcggg cagtccagct ctacagcga ctggtacagc   1020
ccggcctgcg gtaaggctgg ctgccagact tacaagtggg aaaccttcct gaccagcgag   1080
ctgccgcaat ggttgtccgc aacagggcc gtgaagccca ccggcagcgc tgcaatcggc   1140
ttgtcgatgg ccggctcgtc ggcaatgatc ttggccgcct accaccccca gcagttcatc   1200
tacgccggct cgctgtcggc cctgctggac ccctctcagg ggatggggcc tagcctgatc   1260
ggcctcgcga tgggtgacgc cggcggttac aaggccgcag acatgtgggg tccctcgagt   1320
gacccggcat gggagcgcaa cgaccctacg cagcagatcc ccaagctggt cgcaaacaac   1380
acccggctat gggtttattg cgggaacggc accccgaacg agttgggcgg tgccaacata   1440
ccgccgagt tcttggagaa cttcgttcgt agcagcaacc tgaagttcca ggatgcgtac   1500
aacgccgcgg cgggcacaa cgccgtgttc aacttcccgc caacggcac gcacagctgg   1560
</pre>

                                      2

40735WOP00SEQLIST.TXT

```
gagtactggg gcgctcagct caacgccatg aagggtgacc tgcagagttc gttaggcgcc    1620
ggc                                                                  1623
```

```
<210> 4
<211> 726
<212> DNA
<213> Artificial sequence
```

```
<220>
<223> Nucleotide sequence encoding fusion protein
```

```
<400> 4
gaggtgaagc tgcagcagtc tggaactgaa gtggtaaagc ctggggcttc agtgaagttg    60
tcctgcaagg cttctggcta catcttcaca agttatgata tagactgggt gaggcagacg   120
cctgaacagg gacttgagtg gattggatgg atttttcctg gagagggggag tactgaatac   180
aatgagaagt tcaagggcag ggccacactg agtgtagaca agtcctccag cacagcctat   240
atggagctca ctaggctgac atctgaggac tctgctgtct atttctgtgc tagaggggac   300
tactataggc gctactttga cttgtggggc caagggacca cggtcaccgt ctcctcaggt   360
ggaggcggtt caggcggagg tggatccggc ggtggcggat cggacatcca gatgactcag   420
tctccatcat ttctgtctac atctcttgga aacagcatca ccatcacttg ccatgccagt   480
cagaacatca agggttggtt agcctggtac caacaaaagt cagggaatgc tcctcaactg   540
ttgatttata aggcatctag cctgcaatca ggggttccat caagattcag tggcagtgga   600
tctggaacag attatatttt cactatcagc aacctacagc ctgaagatat tgccacttat   660
tactgtcagc attatcaaag ctttccgtgg acgttcggtg agggaccaa gctggaaata   720
aaacgg                                                               726
```

```
<210> 5
<211> 119
<212> PRT
<213> Rat
```

```
<400> 5
Glu Val Lys Leu Gln Gln Ser Gly Thr Glu Val Val Lys Pro Gly Ala
1               5                   10                  15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Ile Phe Thr Ser Tyr
            20                  25                  30
Asp Ile Asp Trp Val Arg Gln Thr Pro Glu Gln Gly Leu Glu Trp Ile
        35                  40                  45
Gly Trp Ile Phe Pro Gly Glu Gly Ser Thr Glu Tyr Asn Glu Lys Phe
    50                  55                  60
Lys Gly Arg Ala Thr Leu Ser Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Thr Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
                85                  90                  95
Ala Arg Gly Asp Tyr Tyr Arg Arg Tyr Phe Asp Leu Trp Gly Gln Gly
            100                 105                 110
Thr Thr Val Thr Val Ser Ser
            115
```

```
<210> 6
<211> 110
<212> PRT
<213> Rat
```

```
<400> 6
Asp Ile Gln Met Thr Gln Ser Pro Ser Phe Leu Ser Thr Ser Leu Gly
1               5                   10                  15
```

40735WOP00SEQLIST.TXT

```
Asn Ser Ile Thr Ile Thr Cys His Ala Ser Gln Asn Ile Lys Gly Trp
        20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Ser Gly Asn Ala Pro Gln Leu Leu Ile
        35                  40                  45
Tyr Lys Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Tyr Ile Phe Thr Ile Ser Asn Leu Gln Pro
65                  70                  75                  80
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Tyr Gln Ser Phe Pro Trp
            85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala
        100                 105                 110
```

<210> 7
<211> 261
<212> PRT
<213> Artificial sequence

<220>
<223> Fusion protein

<400> 7
```
Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
 1               5                   10                  15
Gly Ser Cys Glu Val Lys Leu Gln Gln Ser Gly Thr Glu Val Val Lys
        20                  25                  30
Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Ile Phe
        35                  40                  45
Thr Ser Tyr Asp Ile Asp Trp Val Arg Gln Thr Pro Glu Gln Gly Leu
    50                  55                  60
Glu Trp Ile Gly Trp Ile Phe Pro Gly Glu Gly Ser Thr Glu Tyr Asn
65                  70                  75                  80
Glu Lys Phe Lys Gly Arg Ala Thr Leu Ser Val Asp Lys Ser Ser Ser
            85                  90                  95
Thr Ala Tyr Met Glu Leu Thr Arg Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110
Tyr Phe Cys Ala Arg Gly Asp Tyr Tyr Arg Arg Tyr Phe Asp Leu Trp
        115                 120                 125
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140
Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser
145                 150                 155                 160
Pro Ser Phe Leu Ser Thr Ser Leu Gly Asn Ser Ile Thr Ile Thr Cys
            165                 170                 175
His Ala Ser Gln Asn Ile Lys Gly Trp Leu Ala Trp Tyr Gln Gln Lys
            180                 185                 190
Ser Gly Asn Ala Pro Gln Leu Leu Ile Tyr Lys Ala Ser Ser Leu Gln
        195                 200                 205
Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
    210                 215                 220
Ile Phe Thr Ile Ser Asn Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr
225                 230                 235                 240
Cys Gln His Tyr Gln Ser Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys
            245                 250                 255
Leu Glu Ile Lys Arg
        260
```

40735WOP00SEQLIST.TXT

```
<210> 8
<211> 1623
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence encoding fusion protein

<400> 8
gaggtgaagc tgcagcagtc tggaactgaa gtggtaaagc ctggggcttc agtgaagttg      60
tcctgcaagg cttctggcta catcttcaca agttatgata tagactgggt gaggcagacg     120
cctgaacagg gacttgagtg gattggatgg attttttcctg gagagggggag tactgaatac   180
aatgagaagt tcaagggcag ggccacactg agtgtagaca agtcctccag cacagcctat     240
atggagctca ctaggctgac atctgaggac tctgctgtct atttctgtgc tagagggggac    300
tactataggc gctactttga cttgtggggc caagggacca cggtcaccgt ctcctcaggt     360
ggaggcggtt caggcggagg tggatccggc ggtggcggat cggacatcca gatgactcag     420
tctccatcat ttctgtctac atctcttgga aacagcatca ccatcacttg ccatgccagt     480
cagaacatca agggttggtt agcctggtac caacaaaagt cagggaatgc tcctcaactg     540
ttgatttata aggcatctag cctgcaatca ggggttccat caagattcag tggcagtgga     600
tctggaacag attatatttt cactatcagc aacctacagc ctgaagatat tgccacttat     660
tactgtcagc attatcaaag cttttccgtgg acgttcggtg gagggaccaa gctggaaata     720
aaacggggat atccatcaca ctggcggccg ctcgagcatg catctagatt ctcccggccg     780
gggctgccgg tcgagtacct gcaggtgccg tcgccgtcga tgggccgcga catcaaggtt     840
cagttccaga gcggtgggaa caactcacct gcggtttatc tgctcgacgg cctgcgcgcc     900
caagacgact acaacggctg ggatatcaac accccggcgt tcgagtggta ctaccagtcg     960
ggactgtcga tagtcatgcc ggtcggcggg cagtccagct tctacagcga ctggtacagc    1020
ccggcctgcg gtaaggctgg ctgccagact tacaagtggg aaaccttcct gaccagcgag    1080
ctgccgcaat ggttgtccgc aacagggcc gtgaagccca ccggcagcgc tgcaatcggc     1140
ttgtcgatgg ccggctcgtc ggcaatgatc ttggccgcct accaccccca gcagttcatc    1200
tacgccggct cgctgtcggc cctgctggac ccctctcagg ggatgggggcc tagcctgatc   1260
ggcctcgcga tgggtgacgc cggcggttac aaggccgcag acatgtgggg tccctcgagt    1320
gacccggcat gggagcgcaa cgaccctacg cagcagatcc ccaagctggt cgcaaacaac    1380
acccggctat gggtttattg cgggaacggc accccgaacg agttgggcgg tgccaacata    1440
cccgccgagt tcttggagaa cttcgttcgt agcagcaacc tgaagttcca ggatgcgtac    1500
aacgccgcgg gcgggcacaa cgccgtgttc aacttcccgc ccaacggcac gcacagctgg    1560
gagtactggg gcgctcagct caacgccatg aagggtgacc tgcagagttc gttaggcgcc    1620
ggc                                                                  1623

<210> 9
<211> 5446
<212> DNA
<213> Artificial Sequence

<220>
<223> Plasmid vector generated in a laboratory

<400> 9
gacggatcgg gagatctccc gatccccctat ggtcgactct cagtacaatc tgctctgatg      60
ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg     120
cgagcaaaat ttaagctaca acaggcaag gcttgaccga caattgcatg aagaatctgc       180
ttagggttag gcgttttgcg ctgcttgcg atgtacgggc cagatacg cgttgacatt         240
gattattgac tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata      300
tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc     360
cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc     420
attgacgtca atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt     480
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt     540
atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca     600
```

                                                                                5

40735WOP00SEQLIST.TXT

```
tcgctattac  catggtgatg  cggttttggc  agtacatcaa  tgggcgtgga  tagcggtttg       660
actcacgggg  atttccaagt  ctccacccca  ttgacgtcaa  tgggagtttg  ttttggcacc       720
aaaatcaacg  ggactttcca  aaatgtcgta  acaactccgc  cccattgacg  caaatgggcg       780
gtaggcgtgt  acggtgggag  gtctatataa  gcagagctct  ctggctaact  agagaaccca       840
ctgcttactg  gcttatcgaa  attaatacga  ctcactatag  ggagacccaa  gcttggtacc       900
gagctcggat  ccactagtaa  cggccgccag  tgtgctggaa  ttctgcagat  atccatcaca       960
ctggcggccg  ctcgagcatg  catctagagg  gccctattct  atagtgtcac  ctaaatgcta      1020
gagctcgctg  atcagcctcg  actgtgcctt  ctagttgcca  gccatctgtt  gtttgcccct      1080
cccccgtgcc  ttccttgacc  ctggaaggtg  ccactcccac  tgtcctttcc  taataaaatg      1140
aggaaattgc  atcgcattgt  ctgagtaggt  gtcattctat  tctggggggt  ggggtggggc      1200
aggacagcaa  gggggaggat  tgggaagaca  atagcaggca  tgctggggat  gcggtgggct      1260
ctatggcttc  tgaggcggaa  agaaccagct  ggggctctag  ggggtatccc  cacgcgccct      1320
gtagcggcgc  attaagcgcg  gcgggtgtgg  tggttacgcg  cagcgtgacc  gctacacttg      1380
ccagcgccct  agcgcccgct  cctttcgctt  tcttcccttc  ctttctcgcc  acgttcgccg      1440
gctttccccg  tcaagctcta  aatcggggca  tccctttagg  gttccgattt  agtgctttac      1500
ggcacctcga  ccccaaaaaa  cttgattagg  gtgatggttc  acgtagtggg  ccatcgccct      1560
gatagacggt  ttttcgccct  ttgacgttgg  agtccacgtt  ctttaatagt  ggactcttgt      1620
tccaaactgg  aacaacactc  aaccctatct  cggtctattc  ttttgattta  taagggattt      1680
tggggatttc  ggcctattgg  ttaaaaaatg  agctgattta  acaaaaattt  aacgcgaatt      1740
aattctgtgg  aatgtgtgtc  agttagggtg  tggaaagtcc  ccaggctccc  caggcaggca      1800
gaagtatgca  aagcatgcat  ctcaattagt  cagcaaccag  gtgtggaaag  tccccaggct      1860
ccccagcagg  cagaagtatg  caaagcatgc  atctcaatta  gtcagcaacc  atagtcccgc      1920
ccctaactcc  gcccatcccg  cccctaactc  cgcccagttc  cgcccattct  ccgccccatg      1980
gctgactaat  tttttttatt  tatgcagagg  ccgaggccgc  ctctgcctct  gagctattcc      2040
agaagtagtg  aggaggcttt  tttggaggcc  taggcttttg  caaaaagctc  ccgggagctt      2100
gtatatccat  tttcggatct  gatcaagaga  caggatgagg  atcgtttcgc  atgattgaac      2160
aagatggatt  gcacgcaggt  tctccggccg  cttgggtgga  gaggctattc  ggctatgact      2220
gggcacaaca  gacaatcggc  tgctctgatg  ccgccgtgtt  ccggctgtca  gcgcaggggc      2280
gcccggttct  ttttgtcaag  accgacctgt  ccggtgccct  gaatgaactg  caggacgagg      2340
cagcgcggct  atcgtggctg  gccacgacgg  gcgttccttg  cgcagctgtg  ctcgacgttg      2400
tcactgaagc  gggaagggac  tggctgctat  tgggcgaagt  gccggggcag  gatctcctgt      2460
catctcacct  tgctcctgcc  gagaaagtat  ccatcatggc  tgatgcaatg  cggcggctgc      2520
atacgcttga  tccggctacc  tgcccattcg  accaccaagc  gaaacatcgc  atcgagcgag      2580
cacgtactcg  gatggaagcc  ggtcttgtcg  atcaggatga  tctggacgaa  gagcatcagg      2640
ggctcgcgcc  agccgaactg  ttcgccaggc  tcaaggcgcg  catgcccgac  ggcgaggatc      2700
tcgtcgtgac  ccatggcgat  gcctgcttgc  cgaatatcat  ggtggaaaat  ggccgctttt      2760
ctggattcat  cgactgtggc  cggctgggtg  tggcggaccg  ctatcaggac  atagcgttgg      2820
ctacccgtga  tattgctgaa  gagcttggcg  gcgaatgggc  tgaccgcttc  ctcgtgcttt      2880
acggtatcgc  cgctcccgat  tcgcagcgca  tcgccttcta  tcgccttctt  gacgagttct      2940
tctgagcggg  actctggggt  tcgaaatgac  cgaccaagcg  acgcccaacc  tgccatcacg      3000
agatttcgat  tccaccgccg  ccttctatga  aaggttgggc  ttcggaatcg  ttttccggga      3060
cgccggctgg  atgatcctcc  agcgcgggga  tctcatgctg  gagttcttcg  cccaccccaa      3120
cttgtttatt  gcagcttata  atggttacaa  ataaagcaat  agcatcacaa  atttcacaaa      3180
taaagcattt  ttttcactgc  attctagttg  tggtttgtcc  aaactcatca  atgtatctta      3240
tcatgtctgt  ataccgtcga  cctctagcta  gagcttggcg  taatcatggt  catagctgtt      3300
tcctgtgtga  aattgttatc  cgctcacaat  tccacacaac  atacgagccg  gaagcataaa      3360
gtgtaaagcc  tggggtgcct  aatgagtgag  ctaactcaca  ttaattgcgt  tgcgctcact      3420
gcccgctttc  cagtcgggaa  acctgtcgtg  ccagctgcat  taatgaatcg  gccaacgcgc      3480
ggggagaggc  ggtttgcgta  ttgggcgctc  ttccgcttcc  tcgctcactg  actcgctgcg      3540
ctcggtcgtt  cggctgcggc  gagcggtatc  agctcactca  aaggcggtaa  tacggttatc      3600
cacagaatca  ggggataacg  caggaaagaa  catgtgagca  aaaggccagc  aaaaggccag      3660
gaaccgtaaa  aaggccgcgt  tgctggcgtt  tttccatagg  ctccgccccc  ctgacgagca      3720
tcacaaaaat  cgacgctcaa  gtcagaggtg  cgaaacccg   acaggactat  aaagatacca      3780
ggcgtttccc  cctggaagct  ccctcgtgcg  ctctcctgtt  ccgaccctgc  cgcttaccgg      3840
atacctgtcc  gcctttctcc  cttcgggaag  cgtggcgctt  tctcaatgct  cacgctgtag      3900
gtatctcagt  tcggtgtagg  tcgttcgctc  caagctgggc  tgtgtgcacg  aaccccccgt      3960
tcagcccgac  cgctgcgcct  tatccggtaa  ctatcgtctt  gagtccaacc  cggtaagaca      4020
```

40735WOP00SEQLIST.TXT
```
cgacttatcg ccactggcag cagccactgg taacaggatt agcagagcga ggtatgtagg    4080
cggtgctaca gagttcttga agtggtggcc taactacggc tacactagaa ggacagtatt    4140
tggtatctgc gctctgctga agccagttac cttcggaaaa agagttggta gctcttgatc    4200
cggcaaacaa accaccgctg gtagcggtgg ttttttttgtt tgcaagcagc agattacgcg    4260
cagaaaaaaa ggatctcaag aagatccttt gatcttttct acggggtctg acgctcagtg    4320
gaacgaaaac tcacgttaag ggattttggt catgagatta tcaaaaagga tcttcaccta    4380
gatcctttta aattaaaaat gaagttttaa atcaatctaa agtatatatg agtaaacttg    4440
gtctgacagt taccaatgct taatcagtga ggcacctatc tcagcgatct gtctatttcg    4500
ttcatccata gttgcctgac tccccgtcgt gtagataact acgatacggg agggcttacc    4560
atctggcccc agtgctgcaa tgataccgcg agacccacgc tcaccggctc cagatttatc    4620
agcaataaac cagccagccg gaagggccga gcgcagaagt ggtcctgcaa ctttatccgc    4680
ctccatccag tctattaatt gttgccggga agctagagta agtagttcgc cagttaatag    4740
tttgcgcaac gttgttgcca ttgctacagg catcgtggtg tcacgctcgt cgtttggtat    4800
ggcttcattc agctccggtt cccaacgatc aaggcgagtt acatgatccc ccatgttgtg    4860
caaaaaagcg gttagctcct tcggtcctcc gatcgttgtc agaagtaagt tggccgcagt    4920
gttatcactc atggttatgg cagcactgca taattctctt actgtcatgc catccgtaag    4980
atgcttttct gtgactggtg agtactcaac caagtcattc tgagaatagt gtatgcggcg    5040
accgagttgc tcttgcccgg cgtcaatacg ggataatacc gcgccacata gcagaacttt    5100
aaaagtgctc atcattggaa aacgttcttc ggggcgaaaa ctctcaagga tcttaccgct    5160
gttgagatcc agttcgatgt aacccactcg tgcacccaac tgatcttcag catcttttac    5220
tttcaccagc gtttctgggt gagcaaaaac aggaaggcaa aatgccgcaa aaaagggaat    5280
aagggcgaca cggaaatgtt gaatactcat actcttcctt tttcaatatt attgaagcat    5340
ttatcagggt tattgtctca tgagcggata catatttgaa tgtatttaga aaaataaaca    5400
aataggggtt ccgcgcacat ttccccgaaa agtgccacct gacgtc                    5446
```

```
<210> 10
<211> 14
<212> PRT
<213> Articifical sequence

<220>
<223> Generated in a laboratory from synthetic material

<400> 10
Gly Tyr Pro Ser His Trp Arg Pro Leu Glu His Ala Ser Arg
 1               5                   10

<210> 11
<211> 42
<212> DNA
<213> Artificial sequence

<220>
<223> Generated in a laboratory from synthetic material

<400> 11
ggatatccat cacactggcg gccgctcgag catgcatcta ga                       42

<210> 12
<211> 5538
<212> DNA
<213> Artificial Sequence

<220>
<223> Plasmid vector generated in a laboratory

<400> 12
```

40735WOP00SEQLIST.TXT

```
aacggctccg cccactatta atgaaattaa aaattccaat tttaaaaaac gcagcaagag      60
aaacatttgt atgaaagaat gcgtagaagg aaagaaaaat gtcgtcgaca tgctgaacaa     120
caagattaat atgcctccgt gtataaaaaa aatattgaac gatttgaaag aaaacaatgt     180
accgcgcggc ggtatgtaca ggaagaggtt tatactaaac tgttacattg caaacgtggt     240
ttcgtgtgcc aagtgtgaaa accgatgttt aatcaaggct ctgacgcatt tctacaacca     300
cgactccaag tgtgtgggtg aagtcatgca tcttttaatc aaatcccaag atgtgtataa     360
accaccaaac tgccaaaaaa tgaaaactgt cgacaagctc tgtccgtttg ctggcaactg     420
caagggtctc aatcctattt gtaattattg aataataaaa caattataaa tgctaaattt     480
gttttttatt aacgatacaa accaaacgca acaagaacat ttgtagtatt atctataatt     540
gaaaacgcgt agtataatc gctgaggtaa tatttaaaat cattttcaaa tgattcacag     600
ttaatttgcg acaatataat tttattttca cataaactag acgccttgtc gtcttcttct     660
tcgtattcct tctctttttc attttctcc tcataaaaat taacatagtt attatcgtat     720
ccatatatgt atctatcgta tagagtaaat tttttgttgt cataaatata tatgtctttt     780
ttaatggggt gtatagtacc gctgcgcata gttttttctgt aatttacaac agtgctattt     840
tctggtagtt cttcggagtg tgttgcttta attattaaat ttatataatc aatgaatttg     900
ggatcgtcgg ttttgtacaa tatgttgccg gcatagtacg cagcttcttc tagttcaatt     960
acaccatttt ttagcagcac cggattaaca taactttcca aaatgttgta cgaaccgtta    1020
aacaaaaaca gttcacctcc cttttctata ctattgtctg cgagcagttg tttgttgtta    1080
aaaataacag ccattgtaat gagacgcaca aactaatatc acaaactgga aatgtctatc    1140
aatatatagt tgctgatatc atggagataa ttaaaatgat aaccatctcg caaataaata    1200
agtattttac tgttttcgta acagttttgt aataaaaaaa cctataaata cggatccctg    1260
caggcctcga gttcgaatct agaagatctg gtaccgagct cgaattcccg ggcggccgct    1320
taattaattg atccgggtta ttagtacatt tattaagcgc tagattctgt gcgttgttga    1380
tttacagaca attgttgtac gtattttaat aattcattaa atttataatc tttagggtgg    1440
tatgttagag cgaaaatcaa atgattttca gcgtctttat atctgaattt aaatattaaa    1500
tcctcaatag atttgtaaaa taggtttcga ttagtttcaa acaagggttg ttttttccgaa    1560
ccgatggctg gactatctaa tggattttcg ctcaacgcca caaaacttgc caaatcttgt    1620
agcagcaatc tagctttgtc gatattcgtt tgtgttttgt tttgtaataa aggttcgacg    1680
tcgttcaaaa tattatgcgc ttttgtattt ctttcatcac tgtcgttagt gtacaattga    1740
ctcgacgtaa acacgttaaa taaagcttgg acatatttaa catcgggcgt gttagcttta    1800
ttaggccgat tatcgtcgtc gtcccaaccc tcgtcgttag aagttgcttc cgaagacgat    1860
tttgccatag ccacacgacg cctattaatt gtgtcggcta acacgtccgc gatcaaattt    1920
gtagttgagc ttttttggaat tatttctgat tgcgggcgtt tttgggcggg tttcaatcta    1980
actgtgcccg atttttaattc agacaacacg ttagaaagcg atggtgcagg cggtggtaac    2040
atttcagacg gcaaatctac taatggcggc ggtggtggag ctgatgataa atctaccatc    2100
ggtggaggcg caggcggggc tggcggcgga ggcggaggcg gaggtggtgg cggtgatgca    2160
gacggcggtt taggctcaaa tgtctcttta ggcaacacag tcggcacctc aactattgta    2220
ctggtttcgg gcgccgtttt tggtttgacc ggtctgagac gagtgcgatt ttttttcgtttt    2280
ctaatagctt ccaacaattg ttgtctgtcg tctaaaggtg cagcgggttg aggttccgtc    2340
ggcattggtg gagcgggcgg caattcagac atcgatggtg gtggtggtgg tggaggcgct    2400
ggaatgttag gcacgggaga aggtggtggc ggcggtgccg ccggtataat ttgttctggt    2460
ttagtttgtt cgcgcacgat tgtgggcacc ggcgcaggcg ccgctggctg cacaacggaa    2520
ggtcgtctgc ttcgaggcag cgcttggggt ggtggcaatt caatattata attggaatac    2580
aaatcgtaaa aatctgctat aagcattgta atttcgctat cgtttaccgt gccgatattt    2640
aacaaccgct caatgtaagc aattgtattg taaagagatt gtctcaagct cggatcgatc    2700
ccgcacgccg ataacaagcc ttttcatttt tactacagca ttgtagtggc gagacacttc    2760
gctgtcgtcg cctgatgcgg tattttctcc ttacgcatct gtgcggtatt tcacaccgca    2820
tacgtcaaag caaccatagt acgcgccctg tagcggcgca ttaagcgcgg cgggtgtggt    2880
ggttacgcgc agcgtgaccg ctacacttgc cagcgcccta gcgccgctc ctttcgcttt    2940
cttcccttcc tttctcgcca cgttcgccgg ctttccccgt caagctctaa atcgggggct    3000
ccctttaggg ttccgattta gtgctttacg gcacctcgac cccaaaaaac ttgatttggg    3060
tgatggttca cgtagtgggc catcgccctg atagacggtt tttcgccctt gacgttgga    3120
gtccacgttc tttaatagtg gactcttgtt ccaaactgga acaacactca accctatctc    3180
gggctattct tttgatttat aagggatttt gccgatttcg cctattggt taaaaaatga    3240
gctgatttaa caaaaattta acgcgaattt taacaaaata ttaacgttta caatttttatg    3300
gtgcactctc agtacaatct gctctgatgc cgcatagtta agccagcccc gacacccgcc    3360
aacacccgct gacgcgccct gacgggcttg tctgctcccg gcatccgctt acagacaagc    3420
```

8

40735WOP00SEQLIST.TXT

```
tgtgaccgtc tccgggagct gcatgtgtca gaggtttttca ccgtcatcac cgaaacgcgc       3480
gagacgaaag ggcctcgtga tacgcctatt tttataggtt aatgtcatga taataatggt       3540
ttcttagacg tcaggtggca cttttcgggg aaatgtgcgc ggaacccta  tttgtttatt       3600
tttctaaata cattcaaata tgtatccgct catgagacaa taaccctgat aaatgcttca       3660
ataatattga aaaaggaaga gtatgagtat tcaacatttc cgtgtcgccc ttattccctt       3720
ttttgcggca ttttgccttc ctgtttttgc tcacccagaa acgctggtga aagtaaaaga       3780
tgctgaagat cagttgggtg cacgagtggg ttacatcgaa ctggatctca acagcggtaa       3840
gatccttgag agttttcgcc ccgaagaacg ttttccaatg atgagcactt ttaaagttct       3900
gctatgtggc gcggtattat cccgtattga cgccgggcaa gagcaactcg gtcgccgcat       3960
acactattct cagaatgact tggttgagta ctcaccagtc acagaaaagc atcttacgga       4020
tggcatgaca gtaagagaat tatgcagtgc tgccataacc atgagtgata acactgcggc       4080
caacttactt ctgacaacga tcggaggacc gaaggagcta accgcttttt tgcacaacat       4140
gggggatcat gtaactcgcc ttgatcgttg ggaaccggag ctgaatgaag ccataccaaa       4200
cgacgagcgt gacaccacga tgcctgtagc aatggcaaca acgttgcgca aactattaac       4260
tggcgaacta cttactctag cttcccggca acaattaata gactggatgg aggcggataa       4320
agttgcagga ccacttctgc gctcggccct tccggctggc tggtttattg ctgataaatc       4380
tggagccggt gagcgtgggt ctcgcggtat cattgcagca ctggggccag atggtaagcc       4440
ctcccgtatc gtagttatct acacgacggg gagtcaggca actatggatg aacgaaatag       4500
acagatcgct gagataggtg cctcactgat taagcattgg taactgtcag accaagttta       4560
ctcatatata ctttagattg atttaaaact tcattttaa  tttaaaagga tctaggtgaa       4620
gatcctttt  gataatctca tgaccaaaat cccttaacgt gagttttcgt tccactgagc       4680
gtcagacccc gtagaaaaga tcaaaggatc ttcttgagat ccttttttc  tgcgcgtaat       4740
ctgctgcttg caaacaaaaa aaccaccgct accagcggtg gtttgtttgc cggatcaaga       4800
gctaccaact ctttttccga aggtaactgg cttcagcaga gcgcagatac caaatactgt       4860
ccttctagtg tagccgtagt taggccacca cttcaagaac tctgtagcac cgcctacata       4920
cctcgctctg ctaatcctgt taccagtggc tgctgccagt ggcgataagt cgtgtcttac       4980
cgggttggac tcaagacgat agttaccgga taaggcgcag cggtcgggct gaacgggggg       5040
ttcgtgcaca cagcccagct tggagcgaac gacctacacc gaactgagat acctacagcg       5100
tgagctatga aaagcgcca  cgcttcccga agggagaaag gcggacaggt atccggtaag       5160
cggcagggtc ggaacaggag agcgcacgag ggagcttcca gggggaaacg cctggtatct       5220
ttatagtcct gtcgggtttc gccacctctg acttgagcgt cgattttgt  gatgctcgtc       5280
aggggggcgg agcctatgga aaaacgccag caacgcggcc tttttacggt tcctggcctt       5340
ttgctggcct tttgctcaca tgttctttcc tgcgttatcc cctgattctg tggataaccg       5400
tattaccgcc tttgagtgag ctgataccgc tcgccgcagc cgaacgaccg agcgcagcga       5460
gtcagtgagc gaggaagcgg aagagcgccc aatacgcaaa ccgcctctcc ccgcgcgttg       5520
gccgattcat taatgcag                                                     5538
```

```
<210> 13
<211> 978
<212> DNA
<213> Mycobacterium tuberculosis
```

```
<400> 13
atgacagacg tgagccgaaa gattcgagct tggggacgcc gattgatgat cggcacggca        60
gcggctgtag tccttccggg cctggtgggg cttgccggcg gagcggcaac cgcgggcgcg       120
ttctcccggc cggggctgcc ggtcgagtac ctgcaggtgc cgtcgccgtc gatgggccgc       180
gacatcaagg ttcagttcca gagcggtggg aacaactcac ctgcggttta tctgctcgac       240
ggcctgcgcg cccaagacga ctacaacggc tgggatatca caccccggc  gttcgagtgg       300
tactaccagt cgggactgtc gatagtcatg ccggtcggcg gcagtccag  cttctacagc       360
gactggtaca gcccggcctg cggtaaggct ggctgccaga cttacaagtg gaaaccttc       420
ctgaccagcg agctgccgca atggttgtcc gccaacaggg ccgtgaagcc caccggcagc       480
gctgcaatcg gcttgtcgat ggccggctcg tcggcaatga tcttggccgc ctaccacccc       540
cagcagttca tctacgccgg ctcgctgtcg gccctgctgg acccctctca ggggatgggg       600
cctagcctga tcggcctcgc gatgggtgac gccggcggtt acaaggccgc agacatgtgg       660
ggtccctcga gtgacccggc atgggagcgc aacgacccta cgcagcagat ccccaagctg       720
gtcgcaaaca cacccggct  atgggtttat tgcgggaacg gcaccccgaa cgagttgggc       780
ggtgccaaca tacccgccga gttcttggag aacttcgttc gtagcagcaa cctgaagttc       840
```

40735WOP00SEQLIST.TXT
caggatgcgt acaacgccgc gggcgggcac aacgccgtgt tcaacttccc gcccaacggc   900
acgcacagct gggagtactg gggcgctcag ctcaacgcca tgaagggtga cctgcagagt   960
tcgttaggcg ccggctga   978

<210> 14
<211> 325
<212> PRT
<213> Mycobacterium tuberculosis

<400> 14
Met Thr Asp Val Ser Arg Lys Ile Arg Ala Trp Gly Arg Arg Leu Met
 1   5   10   15
Ile Gly Thr Ala Ala Ala Val Val Leu Pro Gly Leu Val Gly Leu Ala
                20   25   30
Gly Gly Ala Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
            35   40   45
Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Val
        50   55   60
Gln Phe Gln Ser Gly Gly Asn Asn Ser Pro Ala Val Tyr Leu Leu Asp
65   70   75   80
Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr Pro
                85   90   95
Ala Phe Glu Trp Tyr Tyr Gln Ser Gly Leu Ser Ile Val Met Pro Val
            100   105   110
Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala Cys Gly
        115   120   125
Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu Thr Ser Glu
    130   135   140
Leu Pro Gln Trp Leu Ser Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
145   150   155   160
Ala Ala Ile Gly Leu Ser Met Ala Gly Ser Ser Ala Met Ile Leu Ala
                165   170   175
Ala Tyr His Pro Gln Gln Phe Ile Tyr Ala Gly Ser Leu Ser Ala Leu
            180   185   190
Leu Asp Pro Ser Gln Gly Met Gly Pro Ser Leu Ile Gly Leu Ala Met
        195   200   205
Gly Asp Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro Ser Ser
    210   215   220
Asp Pro Ala Trp Glu Arg Asn Asp Pro Thr Gln Gln Ile Pro Lys Leu
225   230   235   240
Val Ala Asn Asn Thr Arg Leu Trp Val Tyr Cys Gly Asn Gly Thr Pro
                245   250   255
Asn Glu Leu Gly Gly Ala Asn Ile Pro Ala Glu Phe Leu Glu Asn Phe
            260   265   270
Val Arg Ser Ser Asn Leu Lys Phe Gln Asp Ala Tyr Asn Ala Ala Gly
        275   280   285
Gly His Asn Ala Val Phe Asn Phe Pro Pro Asn Gly Thr His Ser Trp
    290   295   300
Glu Tyr Trp Gly Ala Gln Leu Asn Ala Met Lys Gly Asp Leu Gln Ser
305   310   315   320
Ser Leu Gly Ala Gly
                325

<210> 15
<211> 826
<212> DNA
<213> Mycobacterium tuberculosis

40735WOP00SEQLIST.TXT

<400> 15
```
tctgctagct tgagtctggt caggcatcgt cgtcagcagc gcgatgcccc tatgtttgtc       60
gtcgactcag atatcgcggc aatccaatct cccgcctgcg ccggcggtgc tgcaaactac      120
tcccggagga atttcgacgt gcgcatcaag atcttcatgc tggtcacggc tgtcgttttg      180
ctctgttgtt cgggtgtcgc cacggccgcg cccaagacct actgcgagga gttgaaaggc      240
accgataccg gccaggcgtg ccagattcaa atgtccgacc cggcctacaa catcaacatc      300
agcctgccca gttactaccc cgaccagaag tcgctggaaa attacatcgc ccagacgcgc      360
gacaagttcc tcagcgcggc cacatcgtcc actccacgcg aagcccccta cgaattgaat      420
atcacctcgg ccacatacca gtccgcgata ccaccgcgtg gtacgcaggc cgtggtgctc      480
aaggtctacc agaacgccgg cggcacgcac ccaacgacca cgtacaaggc cttcgattgg      540
gaccaggcct atcgcaagcc aatcacctat gacacgctgt ggcaggctga caccgatccg      600
ctgccagtcg tcttccccat tgtgcaaggt gaactgagca agcagaccgg acaacaggta      660
tcgatagcgc cgaatgccgg cttggacccg gtgaattatc agaacttcgc agtcacgaac      720
gacggggtga ttttcttctt caacccgggg gagttgctgc ccgaagcagc cggcccaacc      780
caggtattgg tcccacgttc cgcgatcgac tcgatgctgg cctaga                     826
```

<210> 16
<211> 228
<212> PRT
<213> Mycobacterium tuberculosis

<400> 16
```
Met Arg Ile Lys Ile Phe Met Leu Val Thr Ala Val Val Leu Leu Cys
1               5                   10                  15
Cys Ser Gly Val Ala Thr Ala Ala Pro Lys Thr Tyr Cys Glu Glu Leu
            20                  25                  30
Lys Gly Thr Asp Thr Gly Gln Ala Cys Gln Ile Gln Met Ser Asp Pro
        35                  40                  45
Ala Tyr Asn Ile Asn Ile Ser Leu Pro Ser Tyr Tyr Pro Asp Gln Lys
    50                  55                  60
Ser Leu Glu Asn Tyr Ile Ala Gln Thr Arg Asp Lys Phe Leu Ser Ala
65                  70                  75                  80
Ala Thr Ser Ser Thr Pro Arg Glu Ala Pro Tyr Glu Leu Asn Ile Thr
                85                  90                  95
Ser Ala Thr Tyr Gln Ser Ala Ile Pro Pro Arg Gly Thr Gln Ala Val
            100                 105                 110
Val Leu Lys Val Tyr Gln Asn Ala Gly Gly Thr His Pro Thr Thr Thr
            115                 120                 125
Tyr Lys Ala Phe Asp Trp Asp Gln Ala Tyr Arg Lys Pro Ile Thr Tyr
        130                 135                 140
Asp Thr Leu Trp Gln Ala Asp Thr Asp Pro Leu Pro Val Val Phe Pro
145                 150                 155                 160
Ile Val Gln Gly Glu Leu Ser Lys Gln Thr Gly Gln Gln Val Ser Ile
                165                 170                 175
Ala Pro Asn Ala Gly Leu Asp Pro Val Asn Tyr Gln Asn Phe Ala Val
            180                 185                 190
Thr Asn Asp Gly Val Ile Phe Phe Phe Asn Pro Gly Glu Leu Leu Pro
            195                 200                 205
Glu Ala Ala Gly Pro Thr Gln Val Leu Val Pro Arg Ser Ala Ile Asp
    210                 215                 220
Ser Met Leu Ala
225
```

<210> 17
<211> 315
<212> DNA

11

40735WOP00SEQLIST.TXT
<213> Mycobacterium tuberculosis

<400> 17
gattgcggat cccatgacag agcagcagtg gaatttcgcg ggtatcgagg ccgcggcaag   60
cgcaatccag ggaaatgtca cgtccattca ttccctcctt gacgagggga agcagtccct   120
gaccaagctc gcagcggcct ggggcggtag cggttcggag gcgtaccagg gtgtccagca   180
aaaatgggac gccacggcta ccgagctgaa caacgcgctg cagaacctgg cgcggacgat   240
cagcgaagcc ggtcaggcaa tggcttcgac cgaaggcaac gtcactggga tgttcgcata   300
ggaagcttgg gaatc   315

<210> 18
<211> 95
<212> PRT
<213> Mycobacterium tuberculosis

<400> 18
Met Thr Glu Gln Gln Trp Asn Phe Ala Gly Ile Glu Ala Ala Ala Ser
 1   5   10   15
Ala Ile Gln Gly Asn Val Thr Ser Ile His Ser Leu Leu Asp Glu Gly
            20   25   30
Lys Gln Ser Leu Thr Lys Leu Ala Ala Ala Trp Gly Gly Ser Gly Ser
        35   40   45
Glu Ala Tyr Gln Gly Val Gln Gln Lys Trp Asp Ala Thr Ala Thr Glu
    50   55   60
Leu Asn Asn Ala Leu Gln Asn Leu Ala Arg Thr Ile Ser Glu Ala Gly
65   70   75   80
Gln Ala Met Ala Ser Thr Glu Gly Asn Val Thr Gly Met Phe Ala
                85   90   95

1

A.	CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]:   C07K 14/35, 16/28, 16/46, 19/00, C12N 5/20, 15/13, A61K 39/04, 39/395, A61P 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Database: STN. Files: CA, Medline, Biosis. Keywords: single chain, scfv, antibod? immunoglob? antigen presenting cell, dendritic cell, dc, Dec-205, CD11c, NLDC-145, N418, mycobacter? 85b.

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 2002/0187131 A1 (D. HAWIGER et al) 12 December 2002. See entire document.	1-53
X	EP 1046651 A1 (KONINKLIJKE UNIVERSITEIT NIJMEGEN) 25 October 2000. See entire document, especially page 5 line 18 and claim 7.	1-53
X	WO 00/00156 A2 (TRUSTEES OF DARTMOUTH COLLEGE) 6 January 2000. See entire document, especially page 9.	1-53

[X] Further documents are listed in the continuation of Box C	[X] See patent family annex

*	Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2003	**9 JAN 2004**

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**FRANCES RODEN** Telephone No : (02) 6283 2239

C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/45449 A1 (D. HART) 4 December 1997. See entire document.	1-53
X	The Journal of Experimental Medicine, vol. 194, number 6, 2001, pages 769-779, D. HAWIGER et al, "Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo." See entire document, especially pages 770, 771 and 775. Citation available at http://www.jem.org/cgi/content/full/194/6/769	1-53
X	The Journal of Experimental Medicine, vol. 196, number 12, 2002, pages 1627-1638, L. BONIFAZ et al, "Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation of major histocompatibility complex class I products and peripheral $CD8^+$ T cell tolerance." See entire document. Citation available at http://www.jem.org/cgi/doi/10.1084/jem.20021598	1-53
X	WO 99/36507 A1 (GENITRIX LLC) 22 July 1999. See entire document.	28-46
X	WO 01/09186 A2 (MEDAREX, INC.) 8 February 2001. See entire document, especially claim 5 and example 8.	1-27
A	Blood, vol. 90, number 9, 1997, pages 3245-3287, D. HART, "Dendritic cells: Unique leukocyte populations which control the primary immune response." See entire document.	1-53
A	Nature, vol. 375, 1995, pages 151-155, W. JIANG et al, "The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing." See entire document.	1-53
A	The Journal of Immunology, vol. 157, 1996, pages 1406-1414, F. D. FINKELMAN et al, "Dendritic cells can present antigen in vivo in a tolerogenic or immunogenic fashion." See entire document.	1-53

INTERNATIONAL SEARCH REPORT

Box I	Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : **1-53 (in part)**

   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   A full search was not possible on economic grounds. Due to the broad scope of the claims the search was based on the examples described in the specification.

3. ☐ Claims Nos :

   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II	Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member					
EP	1046651	AU	41526/00	CA	2330231	EP	1046651	
		EP	1086137	WO	0063251			
WO	0000156	AU	49548/99					
WO	9745449	AU	29176/97	EP	1015489	NZ	333191	
		US	6432666	US	2003082707			
WO	9936507	AU	22301/99	US	6224870	US	2001031264	
		US	2002131974	US	2003133942			
WO	0109186	AU	62358/00	AU	63835/96	CA	2220461	
		CA	2381565	CN	1203603	EP	0832135	
		EP	1212366	NZ	312328	US	5837243	
		US	6270765	US	6365161	US	6395272	
		US	6410690	US	2002032312	WO	9640789	
US	2002187131	AU	49702/96	CA	2211993	EP	0808366	
		WO	9623882					

END OF ANNEX